PCT WORLD INTELLECTUAL PR
Internation

INTERNATIONAL APPLICATION PUBLISHED UNI WO 9605314A2

(51) International Patent Classification 6 :
C12N 15/32, C07K 14/325, C12Q 1/68, A01N 63/00, C12N 15/82

A2

(11) International Publication Number: WO 96/05314

(43) International Publication Date: 22 February 1996 (22.02.96)

(21) International Application Number: PCT/US95/10310

(22) International Filing Date: 14 August 1995 (14.08.95)

(30) Priority Data:
08/291,368 15 August 1994 (15.08.94) US

(71) Applicant: MYCOGEN CORPORATION [US/US]; 5501 Oberlin Drive, San Diego, CA 92121 (US).

(72) Inventors: PAYNE, Jewel; 2318 Elendil Lane, Davis, CA 95616 (US). SICK, August, J.; 3188 San Helena Drive, Oceanside, CA 92056 (US). NARVA, Kenneth, E.; 12123 Caminito Mira Del Mar, San Diego, CA 92130 (US). SCHNEPF, H., Ernest; 7954 Handel Court, San Diego, CA 92126 (US). SCHWAB, George, E.; 1351 Walnutview, Encinitas, CA 92024 (US).

(74) Agents: SALIWANCHIK, David, R. et al.; Saliwanchik & Saliwanchik, Suite A-1, 2421 N.W. 41st Street, Gainesville, FL 32606-6669 (US).

(81) Designated States: AU, CA, JP, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).

Published
Without international search report and to be republished upon receipt of that report.

(54) Title: PROTEIN TOXINS ACTIVE AGAINST LEPIDOPTERAN PESTS

(57) Abstract

Disclosed and claimed are novel *Bacillus thuringiensis* isolates which have lepidopteran activity. Thus, these isolates, or mutants thereof, can be used to control such insect pests. Further, genes encoding novel δ-endotoxins can be removed from the isolates and transferred to other host microbes, or plants. Expression of the δ-endotoxins in such hosts results in the control of susceptible insect pests in the environment of such hosts.

```
1
MjNNIQNQCV PYNCLxNPEV EILxEERSTG RLPLDISLSL TRFLLSEFVP
GVGVAPGLFD LIWGFITPSx WSLFLLQIEQ LIEQRIETLE RNRAITTLRG
LADSYEbYbE ALREWE-NPN NAQLREDVRI RFANTDDALI TAINNFTLTS
FEIPLLSVYV QAANLHLSLL RDAVSFGQGW GLDIATVNNH YNRLINLIHR

201
YTjHCLDTYN QGLENLRGTN TRQW-RFNQF RRxLTLTVLD IVALFPNYDo
RuYPIQTSSQ LTREIYTSSV IEDSPVSANI PNGFNRAEFG VRPPHLMDFM
NSLFVTAETV RSQTVWGGHL VSSRNTAGH- IMFP.YGVFN PGGAIWIADE
DPRPFYRTLS DPVFVRGGFG zPHYVLGLRG V-FQQTGTNH TRTFRNSGTI

401
DSLDEIPPQD NSGAPWNDYS HVLNHVTFVR WPGEI-GSDS WRAPMFSWTH
RSA--TNoIz Px-ITQIPoV KAH-L-SG-T VVRGPGFTGG DbLRRTz-Go
FA-o-VNI-G -L-QRYRoRI RYASTTzLjb -o-b-G-xb- -GxFxkTMx-
GD-L--xzFx -A-bzToF-F ---QS-FTbG uxuF.SzxEV YIDkbEbbPo

601
-oTFEAE-DK ERAQKAVNAL FTSozQbGbK TzVTzYHIDQ VSNLV-CLSD
EFCLDEKREL SEKVHKAKRL SDxRNLLQDP NFkGINRQoD -GWRGSTDIT
IQxGDDVFKE NYVTLPGTFD ECYPTYLYQK IDESKLK-YT RYQLRGYIED
SQDLEIYLIR YN-KHEpVNV oGTGSLWPLS Vjz-Ix-CGE PNRCAPHLEW

801
NPDL-CSCRD GEKCoHHSHH FSLDIDVGCT DLNEDLzVWb IFKIKTQDGH
ARLGNLEFLE EjPLbGEAL- RVKRAEKKWR DKREKLjLET NIVYKEAKES
VDALFVNSQY DjLQADTNIA MIHAADKRVH RIREAYLPEL SVIPGVNo-I
FEELjGRIFT A--LYDARNV IKNGzFNNGL -CWNVKGHVD VEEQNNHRSV

1001
LVVPEWEAEV SQEVRVCPGR GYILRVTAYK EGYGEGCVTI HEbxNNTDEL
KFSNC-xEjV YPzNTVuCND Y--N-X..-z A--SRNRGYD E-YxSNSSbP
ADYA-VYEEj -YTDGjRzNP CE-NRG...- TPLPAGYVT- ELEYFPETD-
VWbEIGETEG TFIVDSVELL LMEE
```

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AT	Austria	**GB**	United Kingdom	**MR**	Mauritania
AU	Australia	**GE**	Georgia	**MW**	Malawi
BB	Barbados	**GN**	Guinea	**NE**	Niger
BE	Belgium	**GR**	Greece	**NL**	Netherlands
BF	Burkina Faso	**HU**	Hungary	**NO**	Norway
BG	Bulgaria	**IE**	Ireland	**NZ**	New Zealand
BJ	Benin	**IT**	Italy	**PL**	Poland
BR	Brazil	**JP**	Japan	**PT**	Portugal
BY	Belarus	**KE**	Kenya	**RO**	Romania
CA	Canada	**KG**	Kyrgystan	**RU**	Russian Federation
CF	Central African Republic	**KP**	Democratic People's Republic of Korea	**SD**	Sudan
CG	Congo			**SE**	Sweden
CH	Switzerland	**KR**	Republic of Korea	**SI**	Slovenia
CI	Côte d'Ivoire	**KZ**	Kazakhstan	**SK**	Slovakia
CM	Cameroon	**LI**	Liechtenstein	**SN**	Senegal
CN	China	**LK**	Sri Lanka	**TD**	Chad
CS	Czechoslovakia	**LU**	Luxembourg	**TG**	Togo
CZ	Czech Republic	**LV**	Latvia	**TJ**	Tajikistan
DE	Germany	**MC**	Monaco	**TT**	Trinidad and Tobago
DK	Denmark	**MD**	Republic of Moldova	**UA**	Ukraine
ES	Spain	**MG**	Madagascar	**US**	United States of America
FI	Finland	**ML**	Mali	**UZ**	Uzbekistan
FR	France	**MN**	Mongolia	**VN**	Viet Nam
GA	Gabon				

DESCRIPTION

OR-1 ON ORPHAN RECEPTOR BELONGING TO THE NUCLEAR RECEPTOR FAMILY

Cross-Reference to a Related Application

This is a continuation-in-part of co-pending application Serial No. 08/032,778, filed March 16, 1993, which is a continuation of application Serial No. 07/597,607, filed October 15, 1990, now abandoned.

Background of the Invention

The soil microbe *Bacillus thuringiensis* (*B.t.*) is a Gram-positive, spore-forming bacterium characterized by parasporal crystalline protein inclusions. These inclusions often appear microscopically as distinctively shaped crystals. These crystalline proteins can be proforms of δ-endotoxins which are highly toxic to pests and specific in their toxic activity. Certain *B.t.* endotoxin genes have been isolated and sequenced, and recombinant DNA-based *B.t.* products have been produced and approved. In addition, with the use of genetic engineering techniques, new approaches for delivering *B.t.* endotoxins to agricultural environments are under development, including the use of plants genetically engineered with endotoxin genes for insect resistance and the use of stabilized intact microbial cells as *B.t.* endotoxin delivery vehicles (Gaertner, F.H., L. Kim [1988] *TIBTECH* 6:S4-S7). Thus, isolated *B.t.* endotoxin genes are becoming commercially valuable.

Until the last ten years, commercial use of *B.t.* pesticides has been largely restricted to a narrow range of lepidopteran (caterpillar) pests. Preparations of the spores and crystals of *B. thuringiensis* subsp. *kurstaki* have been used for many years as commercial insecticides for lepidopteran pests. For example, *B. thuringiensis* var. *kurstaki* HD-1 produces a delta endotoxin which is toxic to the larvae of a number of lepidopteran insects.

In recent years, however, investigators have discovered *B.t.* pesticides with specificities for a much broader range of pests. For example, subspecies of *B.t.*, namely *israelensis* and *san diego* (a.k.a. *B.t. tenebrionis*, a.k.a. M-7), have been used commercially to control insects of the orders Diptera and Coleoptera, respectively (Gaertner, F.H. [1989] "Cellular Delivery Systems for Insecticidal Proteins: Living and Non-Living Microorganisms," in *Controlled Delivery of Crop Protection Agents*, R.M. Wilkins, ed., Taylor and Francis, New York and London, 1990, pp. 245-255). See also Couch, T.L. (1980) "Mosquito Pathogenicity of *Bacillus thuringiensis* var. *israelensis*," *Developments in Industrial Microbiology* 22:61-76; Beegle, C.C., (1978) "Use of Entomogenous Bacteria in Agroecosystems," *Developments in Industrial Microbiology* 20:97-104. Krieg, A., A.M. Huger, G.A. Langenbruch, W. Schnetter (1983) *Z. ang. Ent.* 96:500-508, describe

a *B.t.* isolate named *Bacillus thuringiensis* var. *tenebrionis*, which is reportedly active against two beetles in the order Coleoptera. These are the Colorado potato beetle, *Leptinotarsa decemlineata*, and *Agelastica alni*.

Recently, new subspecies of *B.t.* have been identified, and genes responsible for active δ-endotoxin proteins have been isolated (Höfte, H., H.R. Whiteley [1989] *Microbiological Reviews* 52(2):242-255). Höfte and Whiteley classified *B.t.* crystal protein genes into 4 major classes. The classes were CryI (Lepidoptera-specific), CryII (Lepidoptera- and Diptera-specific), CryIII (Coleoptera-specific), and CryIV (Diptera-specific). The discovery of strains specifically toxic to other pests has been reported. (Feitelson, J.S., J. Payne, L. Kim [1992] *Bio/Technology* 10:271-275).

The cloning and expression of a *B.t.* crystal protein gene in *Escherichia coli* has been described in the published literature (Schnepf, H.E., H.R. Whiteley [1981] *Proc. Natl. Acad. Sci. USA* 78:2893-2897). U.S. Patent 4,448,885 and U.S. Patent 4,467,036 both disclose the expression of *B.t.* crystal protein in *E. coli*. U.S. Patents 4,797,276 and 4,853,331 disclose *B. thuringiensis* var. *san diego* (a.k.a. *B.t. tenebrionis*, a.k.a. M-7) which can be used to control coleopteran pests in various environments. U.S. Patent No. 5,164,180 discloses a *B.t.* isolate, PS81A2, which is active against lepidopteran pests. U.S. Patent No. 5,151,363 discloses certain isolates of *B.t.* which have activity against nematodes. Many other patents have issued for new *B.t.* isolates and new uses of *B.t.* isolates. The discovery of new *B.t.* isolates and new uses of known *B.t.* isolates remains an empirical, unpredictable art.

Brief Summary of the Invention

The subject invention concerns novel *Bacillus thuringiensis* isolates which have activity against lepidopteran pests.

Specifically, the invention comprises novel *B.t.* isolates and mutants thereof, and novel delta endotoxin genes obtainable from these *B.t.* isolates which encode proteins which are active against lepidopteran pests.

Brief Description of the Drawing

Figure 1 shows the one-letter amino acid sequence of the Generic Formula (SEQ ID NO. 27). Numbering is for convenience and approximate location only. In the Generic Formula, the N-terminal half of the molecule is comprised of residue nos. 1-638. The C-terminal half is comprised of residues 639-1213. Wherein

A = ala	G = gly	M = met	S = ser
C = cys	H = his	N = asn	T = thr
D = asp	I = ile	P = pro	V = val
E = glu	K = lys	Q = gln	W = trp
F = phe	L = leu	R = arg	Y =tyr

k = K or R

z = G, S, D, or N

j = E, Q, R, or K

x = G, S, D, N, E, Q, R, or K

u = C, P, T, or A

b = M, I, L, V, or F

o = C, P, T, A, M, I, L, V, or F

- = any naturally occurring amino acid

. = any naturally occurring amino acid or complete omission thereof.

Brief Description of the Sequences

SEQ ID NO. 1 is the nucleotide sequence of the gene 81A2.

SEQ ID NO. 2 is the amino acid sequence of the toxin 81A2.

SEQ ID NO. 3 is the nucleotide sequence of the gene 91C2.

SEQ ID NO. 4 is the amino acid sequence of the toxin 91C2.

SEQ ID NO. 5 is a radiolabeled oligonucleotide probe used in RFLP analysis as described in Example 3.

SEQ ID NO. 6 is a forward oligonucleotide primer used to amplify gene 91C2 according to the subject invention.

SEQ ID NO. 7 is a reverse oligonucleotide primer used to amplify gene 91C2 according to the subject invention.

SEQ ID NO. 8 is a synthetic oligonucleotide probe used to identify gene 91C2 according to the subject invention.

SEQ ID NO. 9 is the peptide sequence encoded by probes for CryIF genes.

SEQ ID NO. 10 is a nucleotide probe according to the subject invention.

SEQ ID NO. 11 is the peptide sequence encoded by probes for CryIF genes.

SEQ ID NO. 12 is a nucleotide probe according to the subject invention.

SEQ ID NO. 13 is the peptide sequence encoded by probes for CryIF genes.

SEQ ID NO. 14 is a nucleotide probe according to the subject invention.

SEQ ID NO. 15 is the peptide sequence encoded by probes for CryIF genes.

SEQ ID NO. 16 is a nucleotide probe according to the subject invention.

SEQ ID NO. 17 is the peptide sequence encoded by probes for CryIF genes.

SEQ ID NO. 18 is a nucleotide probe according to the subject invention.

SEQ ID NO. 19 is the peptide sequence encoded by probes for CryIF genes.

SEQ ID NO. 20 is a nucleotide probe according to the subject invention.

SEQ ID NO. 21 is the peptide sequence encoded by probes for CryIF genes.

SEQ ID NO. 22 is a nucleotide probe according to the subject invention.

SEQ ID NO. 23 is the peptide sequence encoded by probes for CryIF genes.

SEQ ID NO. 24 is a nucleotide probe according to the subject invention.

SEQ ID NO. 25 is the peptide sequence encoded by probes for CryIF genes.

SEQ ID NO. 26 is a nucleotide probe according to the subject invention.

SEQ ID NO. 27 is the Generic Formula according to the subject invention.

Detailed Disclosure of the Invention

The subject invention concerns isolates of *Bacillus thuringiensis* having anti-lepidopteran activity. These isolates comprise genes which code for δ-endotoxins, which toxins are responsible for the observed anti-lepidopteran activity. Thus, the subject invention concerns anti-lepidopteran *B.t.* isolates, anti-lepidopteran *B.t.* toxins, and genes which encode these toxins. Further embodiments of the subject invention concern recombinant hosts transformed with genes encoding the anti-lepidopteran *B.t.* toxins. The subject invention further concerns methods for controlling lepidopterans, said methods comprising the use of the isolates, toxins, genes, and recombinant hosts of the subject invention.

Specifically exemplified herein are the isolates designated *B.t.* PS81T1, *B.t.* PS53C2, *B.t.* PS31F4, *B.t.* PS86V1, *B.t.* PS 86I2, *B.t.* PS73E, *B.t.* PS81K, *B.t.* PS83E2, *B.t.* PS81E, *B.t.* PS81Z3, *B.t.* PS53B5, *B.t.* PS83R, *B.t.* PS53B2, *B.t.* PS83N2, *B.t.* PS81B5, *B.t.* PS86W1, and *B.t.* PS93C2. Also specifically exemplified is the toxin designated 91C2 and the gene which encodes this toxin. The 91C2 gene is a CryIF gene. CryIF is a subclass of genes within the lepidopteran-active CryI class of *B.t.* genes. The discovery described in the subject application enables a person skilled in the art to identify other CryIF toxins (and genes coding for these toxins) having anti-lepidopteran activity. The toxins of the subject invention are characterized as being active against lepidopterans and having one or more of the following characteristics:

1. A high degree of amino acid homology with toxin 91C2.
2. A nucleotide sequence encoding the toxin wherein the nucleotide sequence hybridizes with probes or genes disclosed herein.
3. A nucleotide sequence encoding the toxin wherein the nucleotide sequence can be amplified by PCR using primers disclosed herein.
4. An amino acid sequence which conforms to the Generic Formula presented herein.
5. Immunoreactivity to an antibody raised to toxin 91C2.

Bacillus thuringiensis isolates useful according to the subject invention have the following characteristics in their biologically pure form:

Table 1. Taxonomic characterization of the *B.t.* isolates of the subject invention

Isolate	Crystal Type	Approx. Toxin MW (kD)	Serotype	Activity
PS81T1	bipyramid	130	aizawai	Lepidoptera
PS53C2	bipyramid	130, 60	kurstaki	Lepidoptera
PS31F4	bipyramid	130, 60	kurstaki	Lepidoptera
PS86V1	bipyramid	130	galleriae	Lepidoptera
PS86I2	bipyramid	130	morrisoni	Lepidoptera
PS73E	bipyramid	130	aizawai	Lepidoptera
PS81K	bipyramid	130	aizawai	Lepidoptera
PS83E2	amorphic	130	aizawai	Lepidoptera
PS81E	bipyramid	130	aizawai	Lepidoptera
PS81Z3	bipyramid	130	aizawai	Lepidoptera
PS53B5	bipyramid	130, 60	kenyae	Lepidoptera
PS83R	bipyramid	130	aizawai	Lepidoptera
PS53B2	bipyramid	130, 60	kenyae	Lepidoptera
PS83N2	bipyramid	130, 60	sotto/kenyae	Lepidoptera
PS81B5	amorphic	130	aizawai	Lepidoptera
PS86W1	bipyramid	130	galleriae	Lepidoptera
PS91C2	bipyramid	130	morrisoni	Lepidoptera

B.t. isolates useful according to the subject invention have been deposited. Also deposited are recombinant microbes comprising the *B.t.* genes of interest. The cultures have been deposited in the permanent collection of the Patent Culture Collection (NRRL), Regional Research Center, 1815 North University Street, Peoria, Illinois 61604 USA.

Culture	Accession Number	Deposit Date
Bacillus thuringiensis PS81IA	NRRL B-18484	April 19, 1989
Bacillus thuringiensis PS91C2	NRRL B-18931	December 27, 1991
E. coli NM522 (pMYC2361)	NRRL B-21016N	December 17, 1992

The subject cultures have been deposited under conditions that assure that access to the cultures will be available during the pendency of this patent application to one determined by the Commissioner of Patents and Trademarks to be entitled thereto under 37 CFR 1.14 and 35 U.S.C. §122. The deposits are available as required by foreign patent laws in countries wherein

counterparts of the subject application, or its pr geny, are filed. However, it should be understood that the availability of a deposit does not constitute a license to practice the subject invention in derogation of patent rights granted by governmental action.

Further, the subject culture deposits will be stored and made available to the public in accord with the provisions of the Budapest Treaty for the Deposit of Microorganisms, i.e., they will be stored with all the care necessary to keep them viable and uncontaminated for a period of at least five years after the most recent request for the furnishing of a sample of the deposit, and in any case, for a period of at least 30 (thirty) years after the date of deposit or for the enforceable life of any patent which may issue disclosing the cultures. The depositor acknowledges the duty to replace the deposits should the depository be unable to furnish a sample when requested, due to the condition of the deposit(s). All restrictions on the availability to the public of the subject culture deposits will be irrevocably removed upon the granting of a patent disclosing them.

Toxins and genes. The toxins and genes according to the subject invention include not only the full length sequences disclosed herein but also fragments of these sequences, longer sequences, and fusion proteins, which retain the characteristic pesticidal activity of the toxins specifically exemplified herein.

One aspect of the subject invention concerns the discovery of a generic chemical formula hereinafter referred to as the Generic Formula (SEQ ID NO. 27), which can be used to identify toxins having activity against lepidopterans. The Generic Formula describes toxin proteins having molecular weights of about 130 kDa.

The Generic Formula is shown in Figure 1 designated by a one-letter amino acid sequence. The Sequence Listing provided herein according to the PatentIn format utilizes the three-letter amino acid code and has no provision for showing a choice between two amino acids at a given position. Therefore, within the PatentIn Sequence Listing, "Xaa" is used to denote points of variation within a sequence, but the single letter code of Figure 1 should be referred to for the specific amino acid substitutions which are acceptable at a given location in the sequence.

Further guidance for characterizing the lepidopteran toxins of the subject invention is provided in Tables 2 and 3, which demonstrate the relatedness among toxins within the known CryI subclasses of lepidopteran toxins. These tables show a numeric score for the best matching alignment between two proteins that reflects: (1) positive scores for exact matches, (2) positive or negative scores reflecting the likelihood (or not) of one amino acid substituting for another in a related protein, and (3) negative scores for the introduction of gaps. A protein sequence aligned to itself will have the highest possible score, i.e., all exact matches and no gaps. However, an unrelated protein or a randomly generated sequence will typically have a low positive score. Related sequences have scores between the random background score and the perfect match score.

The sequence comparisons reported herein were made using the algorithm of Smith and Waterman ([1981] *Advances in Applied Mathematics* 2:482-489), implemented as the program "Bestfit" in the GCG Sequence Analysis Software Package Version 7, April 1991. The sequences were compared with default parameter values (comparison: Swagappep.Cmp, Gap: 3.0, length weight: 0.1). The program output value is referred to as the Quality score.

Tables 2 and 3 show the pairwise alignment scores between the indicated amino acids of the CryI toxin proteins. Table 4 shows the amino acids compared from the proteins of interest.

Table 2 shows the scores prior to adjustment for random sequence scores. Note that for each subclass, the highest alignment score is always with another toxin protein from the same subclass. For example, the highest alignment score with CryIA(a), aside from itself, is with CryIA(d). Furthermore, CryIA(a) scores highest with all three other CryIA toxin proteins. In a similar manner, other CryI toxins score highest with other members of the same subclass. Of particular relevance to the subject invention is the fact that the CryIF toxin proteins score highest with each other.

Table 3 shows the same analysis after subtraction of the average score of 50 alignments of random shuffles of the column sequences with the row sequences. Note that in Table 3 the same relationships hold as in Table 2, i.e., toxin proteins score highest with other members of the same subclass. Again, the two CryIF toxin proteins score highest with each other. Examination of the adjusted alignment scores for members of the same subclass reveals that CryI subclasses can be defined as those proteins with adjusted alignment scores of about 450 or greater.

Thus, certain toxins of the subject invention can be defined as those which have lepidopteran activity and have an alignment value of 450-500 or greater with CryIF(a) or CryIF(b) (91C2). As used herein, the term "alignment value" refers to the adjusted scores obtained above and used to create the scores reported in Table 3.

Table 2. Raw quality scores

	CryIA(a)	CryIA(b)	CryIA(c)	CryIA(d)	CryIB	CryIC	CryID	CryIE(a)	CryIE(b)	CryIF(a)	CryIF(b) (91C2)
CryIA(a)	911	819	706	857	426	519	533	536	535	532	557
CryIA(b)		912	785	790	428	512	540	547	546	543	565
CryIA(c)			914	679	390	482	508	512	533	502	501
CryIA(d)				911	422	514	539	549	538	539	559
CryIB					954	428	410	408	375	421	434
CryIC						926	525	547	480	494	495
CryID							888	505	499	507	497
CryIE(a)								902	722	494	487
CryIE(b)									899	480	477
CryIF(a)										902	803
CryIF(b) (91C2)											900

Table 3. Nct quality scores

	CryIA(a)	CryIA(b)	CryIA(c)	CryIA(d)	CryIB	CryIC	CryID	CryIE(a)	CryIE(b)	CryIF(a)	CryIF(b) (91C2)
CryIA(a)	724	633	520	671	240	332	352	351	350	349	373
CryIA(b)		726	600	606	241	327	359	362	360	360	383
CryIA(c)			728	493	204	295	327	328	347	319	317
CryIA(d)				727	236	328	357	363	353	356	377
CryIB					763	240	229	223	189	235	249
CryIC						738	343	361	293	309	309
CryID							710	325	319	328	318
CryIE(a)								717	538	310	304
CryIE(b)									713	296	294
CryIF(a)										719	620
CryIF(b) (91C2)											713

Table 4.

Protein	Amino acids compared
CryIA(a)	1-607
CryIA(b)	1-608
CryIA(c)	1-609
CryIA(d)	1-607
CryIB	1-636
CryIC	1-617
CryID	1-592
CryIE(a)	1-601
CryIE(b)	1-599
CryIF(a)	1-601
CryIF(b) (91C2)	1-600

Toxins of the subject invention are specifically exemplified herein by the toxin encoded by the gene designated 91C2. Since this toxin is merely exemplary of the toxins of the subject invention, it should be readily apparent that the subject invention further comprises variant toxins (and nucleotide sequences coding for variant toxins) having the same, or essentially the same, biological activity against lepidopterans of 91C2. These equivalent toxins will have amino acid homology with 91C2. This amino acid homology will typically be greater than 75%, preferably be greater than 90%, and most preferably be greater than 95%. The amino acid homology will be highest in certain critical regions of the toxin which account for biological activity or are involved in the determination of three-dimensional configuration which ultimately is responsible for the biological activity. In this regard, certain amino acid substitutions are acceptable and can be readily made in regions which are not critical to activity or are conservative amino acid substitutions which do not affect the three-dimensional configuration of the molecule. For example, amino acids may be placed in the following classes: non-polar, uncharged polar, basic, and acidic. Conservative substitutions whereby an amino acid of one class is replaced with another amino acid of the same type fall within the scope of the subject invention so long as the substitution does not materially alter the biological activity of the compound. Table 5 provides a listing of examples of amino acids belonging to each class.

Table 5

Class of Amino Acid	Examples of Amino Acids
Nonpolar	Ala, Val, Leu, Ile, Pro, Met, Phe, Trp
Uncharged Polar	Gly, Ser, Thr, Cys, Tyr, Asn, Gln
Acidic	Asp, Glu
Basic	Lys, Arg, His

In some instances, non-conservative substitutions can also be made. The critical factor is that these substitutions must not significantly detract from the biological activity of the toxin.

The toxins of the subject invention can also be characterized in terms of the shape and location of toxin inclusions, which are described above.

It should be apparent to a person skilled in this art that genes encoding lepidopteran-active toxins can be identified and obtained through several means. The specific genes exemplified herein may be obtained from the isolates deposited at a culture depository as described above. These genes, or portions or variants thereof, may also be constructed synthetically, for example, by use of a gene machine. As used herein, the terms "variants" or "variations" of genes refer to nucleotide sequences which code for the same toxins or which code for equivalent toxins having lepidopteran activity. Variations of these genes may be readily constructed using standard techniques for making point mutations. Also, fragments of these genes can be made using commercially available exonucleases or endonucleases according to standard procedures. For example, enzymes such as *Bal*31 or site-directed mutagenesis can be used to systematically cut off nucleotides from the ends of these genes. Also, genes which code for active fragments may be obtained using a variety of other restriction enzymes. Proteases may be used to directly obtain active fragments of these toxins.

Equivalent toxins and/or genes encoding these equivalent toxins can also be located from *B.t.* isolates and/or DNA libraries using the teachings provided herein. There are a number of methods for obtaining the pesticidal toxins of the instant invention. For example, antibodies to the pesticidal toxins disclosed and claimed herein can be used to identify and isolate other toxins from a mixture of proteins. Specifically, antibodies may be raised to the portions of the toxins which are most constant and most distinct from other *B.t.* toxins. These antibodies can then be used to specifically identify equivalent toxins with the characteristic activity by immunoprecipitation, enzyme linked immunosorbent assay (ELISA), or Western blotting. Antibodies to the toxins disclosed herein, or to equivalent toxins, or fragments of these toxins, can

readily be prepared using standard procedures in this art. The genes coding for these toxins can then be obtained from the microorganism.

A further method for identifying the toxins and genes of the subject invention is through the use of oligonucleotide probes. These probes are detectable nucleotide sequences. These sequences may be detectable by virtue of an appropriate label or may be made inherently fluorescent as described in International Patent Application No. WO93/16094. As is well known in the art, if the probe molecule and nucleic acid sample hybridize by forming a strong bond between the two molecules, it can be reasonably assumed that the probe and sample have substantial homology. Detection of the probe provides a means for determining in a known manner whether hybridization has occurred. Such a probe analysis provides a rapid method for identifying toxin-encoding genes of the subject invention.

The nucleotide segments which are used as probes according to the invention can be synthesized by use of DNA synthesizers using standard procedures. In the use of labeled nucleotide segments as probes, the particular probe is labeled with any suitable label known to those skilled in the art, including radioactive and non-radioactive labels. Typical radioactive labels include ^{32}P, ^{125}I, ^{35}S, or the like. A probe labeled with a radioactive isotope can be constructed from a nucleotide sequence complementary to the DNA sample by a conventional nick translation reaction, using a DNase and DNA polymerase. The probe and sample can then be combined in a hybridization buffer solution and held at an appropriate temperature until annealing occurs. Preferably, hybridization is conducted under stringent conditions by techniques well known in the art, as described, for example, in Keller, G.H., M.M. Manak (1989) *DNA Probes*, Stockton Press, New York, NY, pp. 169-170. Thereafter, the membrane is washed free of extraneous materials, leaving the sample and bound probe molecules typically detected and quantified by autoradiography and/or liquid scintillation counting.

Non-radioactive labels include, for example, ligands such as biotin or thyroxine, as well as enzymes such as hydrolases or perixodases, or the various chemiluminescers such as luciferin, or fluorescent compounds like fluorescein and its derivatives. The probe may also be labeled at both ends with different types of labels for ease of separation, as, for example, by using an isotopic label at the end mentioned above and a biotin label at the other end.

Duplex formation and stability depend on substantial complementarity between the two strands of a hybrid; a certain degree of mismatch can be tolerated. Therefore, the probes of the subject invention include mutations (both single and multiple), deletions, insertions of the described sequences, and combinations thereof, wherein said mutations, insertions and deletions permit formation of stable hybrids with the target polynucleotide of interest. Mutations, insertions, and deletions can be produced in a given polynucleotide sequence in many ways, and these methods are known to an ordinarily skilled artisan. Other methods may become known in the future.

The known methods include, but are not limited to:

(1) synthesizing chemically or otherwise an artificial sequence which is a mutation, insertion or deletion of the known sequence;

(2) using a probe of the present invention to obtain via hybridization a new sequence or a mutation, insertion or deletion of the probe sequence; and

(3) mutating, inserting or deleting a test sequence *in vitro* or *in vivo*.

It is important to note that the mutational, insertional, and deletional variants generated from a given probe may be more or less efficient than the original probe. Notwithstanding such differences in efficiency, these variants are within the scope of the present invention.

Thus, mutational, insertional, and deletional variants of the disclosed sequences can be readily prepared by methods which are well known to those skilled in the art. These variants can be used in the same manner as the instant probes so long as the variants have substantial sequence homology with the probes. As used herein, substantial sequence homology refers to homology which is sufficient to enable the variant to function in the same capacity as the original probe. Preferably, this homology is greater than 50%; more preferably, this homology is greater than 75%; and most preferably, this homology is greater than 90%. The degree of homology needed for the variant to function in its intended capacity will depend upon the intended use of the sequence. It is well within the skill of a person trained in this art to make mutational, insertional, and deletional mutations which are designed to improve the function of the sequence or otherwise provide a methodological advantage.

Specific nucleotide probes useful according to the subject invention in the rapid identification of CryIF class toxin genes include:

(i) DNA coding for a peptide sequence "Ser Thr Gly Arg Leu Pro Leu Asp" (SEQ ID NO. 9). A specific example of such a probe is "AGTACWGGMA GRTTACCRTT RGAY" (SEQ ID NO. 10);

(ii) DNA coding for a peptide sequence "Glu Asp Ser Pro Val Ser Ala Asn" (SEQ ID NO. 11). A specific example of such a probe is "GARGATTCWC CAGTWTCWGC WAAT" (SEQ ID NO. 12);

(iii) DNA coding for a peptide sequence "Asn Gly Phe Asn Arg Ala Glu Phe Gly Val" (SEQ ID NO. 13). A specific example of such a probe is "AATGGWTTTA ATAGTGCTGA ATTTGGGAGT W" (SEQ ID NO. 14);

(iv) DNA coding for a peptide sequence "Val Thr Ala Glu Thr Val Arg Ser Gln Thr" (SEQ ID NO. 15). A specific example of such a probe is "GTAACWGCAG ARACWGTWAG WAGTCAAACW" (SEQ ID NO. 16);

(v) DNA coding for a peptide sequence "Val Phe Asn Pro Gly Gly Ala Ile Trp Ile Ala Asp Glu" (SEQ ID NO. 17). A specific example of such a probe is

"GTMTTYAATC CWGGWGGMGC MATWTGGATW GCWGATGARG AT" (SEQ ID NO. 18);

(vi) DNA coding for a peptide sequence "Val Arg Gly Gly Phe Gly" (SEQ ID NO. 19). A specific example of such a probe is "GTMMGAGGWG GWTTTGGR" (SEQ ID NO. 20);

(vii) DNA coding for a peptide sequence "Gly Thr Asn His Thr Arg Thr" (SEQ ID NO. 21). A specific example of such a probe is "GGWACRAAYC AYACMMGAAC W" (SEQ ID NO. 22);

(viii) DNA coding for a peptide sequence "Val Arg Trp Pro Gly Glu Ile" (SEQ ID NO. 23). A specific example of such a probe is "GTWMGATGGC CWGGWGARAT W" (SEQ ID NO. 24);

(ix) DNA coding for a peptide sequence "Ser Asp Ser Trp Arg Ala" (SEQ ID NO. 25). A specific example of such a probe is "AGTGATTCWT GGAGAGCW" (SEQ ID NO. 26).

Because of the redundancy of the genetic code, i.e., more than one coding nucleotide triplet (codon) can be used for most of the amino acids used to make proteins, different nucleotide sequences can code for a particular amino acid. Thus, the amino acid sequences of the *B.t.* toxins and peptides can be prepared by equivalent nucleotide sequences encoding the same amino acid sequence of the protein or peptide. Accordingly, the subject invention includes such equivalent nucleotide sequences. Also, inverse or complement sequences are an aspect of the subject invention and can be readily used by a person skilled in this art.

Recombinant hosts. The toxin-encoding genes harbored by the isolates of the subject invention can be introduced into a wide variety of microbial or plant hosts. Expression of the toxin gene results, directly or indirectly, in the intracellular production and maintenance of the pesticide. With suitable microbial hosts, e.g., *Pseudomonas*, live microbes can be applied to the situs of lepidopterans where they will proliferate and be ingested by the pest. The result is a control of this pest. Alternatively, the microbe hosting the toxin gene can be treated under conditions that prolong the activity of the toxin and stabilize the cell. The treated cell, which retains the toxic activity, then can be applied to the environment of the target pest.

Where the *B.t.* toxin gene is introduced via a suitable vector into a microbial host, and said host is applied to the environment in a living state, it is essential that certain host microbes be used. For example, microorganism hosts can be selected which are known to occupy the soil. These microorganisms are selected so as to be capable of successfully competing in the soil with the wild-type microorganisms. It is also important that they provide for stable maintenance and expression of the gene expressing the polypeptide pesticide, and, desirably, provide for improved protection of the pesticide from environmental degradation and inactivation.

A large number of microorganisms are known to inhabit the rhizosphere (the soil surrounding plant roots). These microorganisms include bacteria, algae, and fungi. Of particular interest are microorganisms, such as bacteria, e.g., genera *Bacillus, Pseudomonas, Erwinia, Serratia, Klebsiella, Xanthomonas, Streptomyces, Rhizobium, Rhodopseudomonas, Methylophilius, Agrobacterium, Acetobacter, Lactobacillus, Arthrobacter, Azotobacter, Leuconostoc, Alcaligenes* and *Clostridium*; fungi, particularly yeast, e.g., genera *Saccharomyces, Cryptococcus, Kluyveromyces, Sporobolomyces, Rhodotorula*, and *Aureobasidium*; microalgae, e.g., families *Cyanophyceae, Prochlorophyceae, Rhodophyceae, Dinophyceae, Chrysophyceae, Prymnesiophyceae, Xanthophyceae, Raphidophyceae, Bacillariophyceae, Eustigmatophyceae, Cryptophyceae, Euglenophyceae, Prasinophyceae*, and *Chlorophyceae*. Of particular interest are such phytosphere bacterial species as *Pseudomonas syringae, Pseudomonas fluorescens, Serratia marcescens, Acetobacter xylinum, Agrobacterium tumefaciens, Rhodopseudomonas spheroides, Xanthomonas campestris, Rhizobium melioti, Alcaligenes entrophus*, and *Azotobacter vinlandii*; and phytosphere yeast species such as *Rhodotorula rubra, R. glutinis, R. marina, R. aurantiaca, Cryptococcus albidus, C. diffluens, C. laurentii, Saccharomyces rosei, S. pretoriensis, S. cerevisiae, Sporobolomyces roseus, S. odorus, Kluyveromyces veronae*, and *Aureobasidium pollulans*. Of particular interest are the pigmented microorganisms.

A wide variety of ways are available for introducing a *B.t.* gene encoding a toxin into a microorganism host under conditions which allow for stable maintenance and expression of the gene. These methods are well known to those skilled in the art and are described, for example, in United States Patent No. 5,135,867, which is incorporated herein by reference.

Treatment of cells. As mentioned above, *B.t.* or recombinant cells expressing a *B.t.* toxin can be treated to prolong the toxin activity and stabilize the cell. The pesticide microcapsule that is formed comprises the *B.t.* toxin within a cellular structure that has been stabilized and will protect the toxin when the microcapsule is applied to the environment of the target pest. Suitable host cells may include either prokaryotes or eukaryotes, normally being limited to those cells which do not produce substances toxic to higher organisms, such as mammals. However, organisms which produce substances toxic to higher organisms could be used, where the toxic substances are unstable or the level of application sufficiently low as to avoid any possibility of toxicity to a mammalian host. As hosts, of particular interest will be the prokaryotes and the lower eukaryotes, such as fungi.

The cell will usually be intact and be substantially in the proliferative form when treated, rather than in a spore form, although in some instances spores may be employed.

Treatment of the microbial cell, e.g., a microbe containing the *B.t.* toxin gene, can be by chemical or physical means, or by a combination of chemical and/or physical means, so long as the technique does not deleteriously affect the properties of the toxin, nor diminish the cellular capability of protecting the toxin. Examples of chemical reagents are halogenating agents,

particularly halogens of atomic no. 17-80. More particularly, iodine can be used under mild conditions and for sufficient time to achieve the desired results. Other suitable techniques include treatment with aldehydes, such as formaldehyde and glutaraldehyde; anti-infectives, such as zephiran chloride and cetylpyridinium chloride; alcohols, such as isopropyl and ethanol; various histologic fixatives, such as Lugol iodine, Bouin's fixative, and Helly's fixative (See: Humason, Gretchen L., *Animal Tissue Techniques*, W.H. Freeman and Company, 1967); or a combination of physical (heat) and chemical agents that preserve and prolong the activity of the toxin produced in the cell when the cell is administered to the host's environment. In one preferred embodiment, acids can be used to stabilize the cells. Examples of physical means are short wavelength radiation such as gamma-radiation and X-radiation, freezing, UV irradiation, lyophilization, and the like. Methods for treatment of microbial cells are disclosed in United States Patent Nos. 4,695,455 and 4,695,462, which are incorporated herein by reference.

The cells generally will have enhanced structural stability which will enhance resistance to environmental conditions. Where the pesticide is in a proform, the method of cell treatment should be selected so as not to inhibit processing of the proform to the mature form of the pesticide by the target pest pathogen. For example, formaldehyde will crosslink proteins and could inhibit processing of the proform of a polypeptide pesticide. The method of treatment should retain a substantial portion of the bio-availability or bioactivity of the toxin.

Characteristics of particular interest in selecting a host cell for purposes of production include ease of introducing the *B.t.* gene into the host, availability of expression systems, efficiency of expression, stability of the pesticide in the host, and the presence of auxiliary genetic capabilities. Characteristics of interest for use as a pesticide microcapsule include protective qualities for the pesticide, such as thick cell walls, pigmentation, and intracellular packaging or formation of inclusion bodies; survival in aqueous environments; lack of mammalian toxicity; attractiveness to pests for ingestion; ease of killing and fixing without damage to the toxin; and the like. Other considerations include ease of formulation and handling, economics, storage stability, and the like.

Growth of cells. The cellular host containing the *B.t.* insecticidal gene may be grown in any convenient nutrient medium, where the DNA construct provides a selective advantage, providing for a selective medium so that substantially all or all of the cells retain the *B.t.* gene. These cells may then be harvested in accordance with conventional ways. Alternatively, the cells can be treated prior to harvesting.

The *B.t.* cells of the invention can be cultured using standard art media and fermentation techniques. Upon completion of the fermentation cycle the bacteria can be harvested by first separating the *B.t.* spores and crystals from the fermentation broth by means well known in the art. The recovered *B.t.* spores and crystals can be formulated into a wettable powder, liquid concentrate, granules or other formulations by the addition of surfactants, dispersants, inert

carriers, and ther components to facilitate handling and application for particular target pests. These formulations and application procedures are all well known in the art.

Formulations. Formulated bait granules containing an attractant and spores and crystals of the *B.t.* isolates, or recombinant microbes comprising the gene(s) obtainable from the *B.t.* isolates disclosed herein, can be applied to the soil. Formulated product can also be applied as a seed-coating or root treatment or total plant treatment at later stages of the crop cycle.

As would be appreciated by a person skilled in the art, the pesticidal concentration will vary widely depending upon the nature of the particular formulation, particularly whether it is a concentrate or to be used directly. The pesticide will be present in at least 1% by weight and may be 100% by weight. The dry formulations will have from about 1-95% by weight of the pesticide while the liquid formulations will generally be from about 1-60% by weight of the solids in the liquid phase. The formulations will generally have from about 10^2 to about 10^4 cells/mg. These formulations will be administered at about 50 mg (liquid or dry) to 1 kg or more per hectare.

The formulations can be applied to the environment of the lepidopteran, e.g., soil, by spraying, dusting, sprinkling, or the like.

Mutants. Mutants of the novel isolates of the invention can be made by procedures well known in the art. For example, an asporogenous mutant can be obtained through ethylmethane sulfonate (EMS) mutagenesis of a novel isolate. The mutants can be made using ultraviolet light and nitrosoguanidine by procedures well known in the art.

A smaller percentage of the asporogenous mutants will remain intact and not lyse for extended fermentation periods; these strains are designated lysis minus (-). Lysis minus strains can be identified by screening asporogenous mutants in shake flask media and selecting those mutants that are still intact and contain toxin crystals at the end of the fermentation. Lysis minus strains are suitable for a cell fixation process that will yield a protected, encapsulated toxin protein.

To prepare a phage resistant variant of said asporogenous mutant, an aliquot of the phage lysate is spread onto nutrient agar and allowed to dry. An aliquot of the phage sensitive bacterial strain is then plated directly over the dried lysate and allowed to dry. The plates are incubated at 30°C. The plates are incubated for 2 days and, at that time, numerous colonies could be seen growing on the agar. Some of these colonies are picked and subcultured onto nutrient agar plates. These apparent resistant cultures are tested for resistance by cross streaking with the phage lysate. A line of the phage lysate is streaked on the plate and allowed to dry. The presumptive resistant cultures are then streaked across the phage line. Resistant bacterial cultures show no lysis anywhere in the streak across the phage line after overnight incubation at 30°C. The resistance to phage is then reconfirmed by plating a lawn of the resistant culture onto a nutrient agar plate. The sensitive strain is also plated in the same manner to serve as the positive control. After drying, a drop of the phage lysate is plated in the center of the plate and allowed to dry.

Resistant cultures showed no lysis in the area where the phage lysate has been placed after incubation at 30°C for 24 hours.

Following are examples which illustrate procedures, including the best mode, for practicing the invention. These examples should not be construed as limiting. All percentages are by weight and all solvent mixture proportions are by volume unless otherwise noted.

Example 1 – Culturing of the *B.t.* Isolates of the Invention

A subculture of a novel *B.t.* isolate, or mutants thereof, can be used to inoculate the following medium, a peptone, glucose, salts medium.

Bacto Peptone	7.5 g/l
Glucose	1.0 g/l
KH_2PO_4	3.4 g/l
K_2HPO_4	4.35 g/l
Salt Solution	5.0 ml/l
$CaCl_2$ Solution	5.0 ml/l

Salts Solution (100 ml)

$MgSO_4 \cdot 7H_2O$	2.46 g
$MnSO_4 \cdot H_2O$	0.04 g
$ZnSO_4 \cdot 7H_2O$	0.28 g
$FeSO_4 \cdot 7H_2O$	0.40 g

$CaCl_2$ Solution (100 ml)

$CaCl_2 \cdot 2H_2O$	3.66 g
pH 7.2	

The salts solution and $CaCl_2$ solution are filter-sterilized and added to the autoclaved and cooked broth at the time of inoculation. Flasks are incubated at 30°C on a rotary shaker at 200 rpm for 64 hr.

The above procedure can be readily scaled up to large fermentors by procedures well known in the art.

The *B.t.* spores and/or crystals, obtained in the above fermentation, can be isolated by procedures well known in the art. A frequently-used procedure is to subject the harvested fermentation broth to separation techniques, e.g., centrifugation.

Example 2 – Activity of *B.t.* Isolates Against Lepidopterans

The following strains have been tested for anti-lepidopteran activity with the following results:

Table 6. Bioassay results

	% Mortality	
Strain	*Trichoplusia ni*	*Spodoptera exigua*
PS81T1	96, 8	
PS53C2	100, 100	
PS31F4		100, 100
PS86V1	100	
PS86I2	100, 92	
PS73E	100, 100	
PS81K	100, 100	
PS83E2	100, 100	
PS81E	100, 92	
PS81Z3	100	
PS53B5		100
PS83R	100	
PS53B2		100
PS83N2	100	
PS81B5	100, 100	
PS86W1	100	
PS91C2	100, 100	
PS81A2	100, 100	

Spodoptera exigua bioassay procedure. *B.t.* cultures were harvested and resuspended in sterile deionized water. Fixed volumes of each culture were incorporated into USDA Insect Diet (Technical Bulletin 1528, U.S. Department of Agriculture, 1976). Twenty-four neonate *S. exigua* were exposed to the diet for 6 days. Mortality readings were taken at this time.

Trichoplusia ni bioassay procedure. *B.t.* cultures were harvested and resuspended in sterile deionized water. Fixed volumes of each culture were top loaded onto USDA Insect Diet. Trays were infested with neonate *T. ni*. After 6 days mortality was determined.

Example 3 – Characterization of Toxin Genes by RFLP Analysis

Total cellular DNA was prepared from *Bacillus thuringiensis* (*B.t.*) cells grown to an optical density, at 600 nm, of 1.0. The cells were recovered by centrifugation, and protoplasts were prepared in TES buffer (30 mM Tris-HCl, 10 mM EDTA, 50 mM NaCl, pH = 8.0) containing 20% sucrose and 50 mg/ml lysozyme. The protoplasts were lysed by addition of SDS to a final concentration of 4%. The cellular material was precipitated overnight at 4°C in 100 mM

(final concentration) neutral potassium chloride. The supernate was extracted twice with phenol/chloroform (1:1). The DNA was precipitated with ethanol and purified by isopycnic banding on a cesium chloride-ethidium bromide gradient.

Total cellular DNA isolated from *B.t.* cells was digested with a restriction endonuclease and separated by electrophoresis on a 0.8% (w/v) agarose-TAE (50 mM Tris-HCl, 20 mM NaOAc, 2.5 mM EDTA, pH = 8.0) buffered gel. A Southern blot of the gel was hybridized with the [^{32}P]-radiolabeled oligonucleotide probe, ATGATTCATGCGGCAGATA (SEQ ID NO. 5), and then washed to remove unbound radioactivity. The blot was exposed to KODAK X-OMAT™ film using standard autoradiography techniques. The results are an array of hybridizing bands (fingerprint) which correspond to toxin genes or toxin gene fragments. This type of characterization is known as Restriction Fragment Length Polymorphism (RFLP) analysis which classifies each isolate by a distinct DNA fingerprint.

Table 7. DNA fingerprints for *B.t.* isolates of the subject invention

Isolate	Hybridizing *Hin*dIII Fragments (Kb)
PS81T1	1.13, 3.0, 9.4
PS53C2	1.052, 5.8, 6.6
PS31F4	5.5, 8.0
PS86V1	5.5, 6.0, 6.6
PS86I2	5.0, 6.6, 7.5, 12
PS73E	1.052, 1.13, 3.0, 8.5
PS81K	3.2, 7.5, 9.4, 13
PS83E2	3.2, 8.5, 12
PS81E	1.13, 3.2, 9.4
PS81Z3	1.13, 3.0, 8.5
PS53B5	1.13, 3.0, 7.5
PS83R	1.13, 3.0, 8.5, 12
PS53B2	1.052, 1.13, 3.0, 7.5
PS83N2	5.5
PS81B5	8.0, 13
PS86W1	5.5, 6.6
PS91C2	1.13, 3.0, 6.0, 7.5, 8.5
PS81A2	13, 16

Table 8. Hybridizing *Hin*dIII fragments of *B.t.* isolates of the subject invention

Isolate	Novel Hybridizing *Hin*dIII Fragments (~Kb)
PS91C2	3.0, 6.0, 7.5
PS83E2	3.2
PS86I2	5.0
PS31F4	5.5, 8.0
PS53C2	5.8
PS81T1	9.4

Example 4 – Molecular Cloning and Expression of a Novel *Cry*IF Toxin Gene from *Bacillus thuringiensis* Strain PS91C2

Total cellular DNA was prepared from *Bacillus thuringiensis* (*B.t.*) cells grown to an optical density, at 600 nm, of 1.0. Cells were pelleted by centrifugation and resuspended in protoplast buffer (20 mg/ml lysozyme in 0.3 M sucrose, 25 mM Tris-Cl [pH 8.0], 25 mM EDTA). After incubation at 37°C for 1 hour, protoplasts were lysed by two cycles of freezing and thawing. Nine volumes of a solution of 0.1 M NaCl, 0.1% SDS, 0.1 M Tris-Cl were added to complete lysis. The cleared lysate was extracted twice with phenol:chloroform (1:1). Nucleic acids were precipitated with two volumes of ethanol and pelleted by centrifugation. The pellet was resuspended in TE buffer and RNase was added to a final concentration of 50 μg/ml. After incubation at 37°C for 1 hour, the solution was extracted once each with phenol:chloroform (1:1) and TE-saturated chloroform. DNA was precipitated from the aqueous phase by the addition of one-tenth volume of 3 M NaOAc and two volumes of ethanol. DNA was pelleted by centrifugation, washed with 70% ethanol, dried, and resuspended in TE buffer.

A 1.58 kbp fragment of the novel 130 kDa toxin gene was obtained by polymerase chain reaction (PCR) amplification from PS91C2 cellular DNA using the following primers: forward 5'-GAGTGGGAAG CAGATCTTAA TAATGCACAA TTAAGG-3' (SEQ ID NO. 6) and reverse 5'-ATAC(C or T)CGATCGATATGATA(G or A)TCCGT-3' (SEQ ID NO. 7). This DNA fragment was cloned into pBluescript S/K (Stratagene, La Jolla, CA) and the DNA sequence determined by dideoxynucleotide sequencing methodology (Sanger *et al.* [1977] *Proc. Natl. Acad. Sci. USA* 74:5463-5467) using Sequenase (U.S. Biochemicals, Cleveland, OH). DNA sequences unique to the *Cry*IF gene were identified by computer comparison with other *Cry*I genes. An oligonucleotide probe with the following sequence was synthesized: 5'-CCCAATGTGAATGTACTTTGCGC-3' (SEQ ID NO. 8). This probe was radiolabeled with ^{32}P

and used in standard hybridizations of Southern blots of PS91C2 total cellular DNA. Hybridizing bands included an approximately 7.5 kbp *Hin*dIII fragment.

A gene library was constructed from PS91C2 DNA partially digested with *Nde*II. Partial restriction digests were fractionated by agarose gel electrophoresis. DNA fragments 9.3 to 23 kbp in size were excised from the gel, electroeluted from the gel slice, purified on an Elutip D ion exchange column (Schleicher and Schuell, Keene, NH), and recovered by ethanol precipitation. The *Nde*II inserts were ligated into *Bam*HI-digested LambdaGem-11 (Promega, Madison, WI). Recombinant phage were packaged and plated on *E. coli* KW251 cells. Plaques were screened by hybridization with each of the respective probes described above. Hybridizing phage were plaque-purified and used to infect liquid cultures of *E. coli* KW251 cells for isolation of DNA by standard procedures (Maniatis *et al., supra*).

For subcloning the gene encoding the 130 kDa *Cry*IF toxin, preparative amounts of phage DNA were digested with *Sau*3A and electrophoresed on agarose gel. The approximately 8 kbp band containing the toxin gene was excised from the gel, electroeluted from the gel slice, and purified by ion exchange chromatography as described above. The purified DNA insert was ligated into an *Xho*I-digested pHTBlueII (an *E. coli/B. thuringiensis* shuttle vector comprised of pBluescript S/K (Stratagene) and the replication origin from a resident *B.t.* plasmid (D. Lereclus *et al.* [1989] *FEMS Microbiol. Lett.* 60:211-218). The ligation mix was used to transform frozen, competent *E. coli* NM522 cells (ATCC 47000). β-galactosidase transformants were screened by restriction digestion of alkaline lysate plasmid minipreps as above. The desired plasmid construct, pMYC2361, contains a toxin gene that is novel compared to other toxin genes containing insecticidal proteins.

pMYC2361 was introduced into the acrystalliferous (Cry^-) *B.t.* host, CryB (A. Aronson, Purdue University, West Lafayette, IN) by electroporation. Expression of the 130 kDa toxin was demonstrated by SDS-PAGE analysis. NaBr-purified crystals were prepared (Pfannenstiel, M.A. *et al.* [1984] *FEMS Microbiol. Lett.* 21:39) for determination of toxicity of the cloned gene product to *Plutella xylostella* by the screening method described in Example 3. The LC_{50} for the *Cry*IF toxin against *P. xylostella* was determined to be 5 μg toxin/ml diet.

Example 5 – Insertion of Toxin Genes Into Plants

One aspect of the subject invention is the transformation of plants with genes encoding a lepidopteran toxin. The transformed plants are resistant to attack by lepidopterans.

Genes encoding lepidopteran-active toxins, as disclosed herein, can be inserted into plant cells using a variety of techniques which are well known in the art. For example, a large number of cloning vectors comprising a replication system in *E. coli* and a marker that permits selection of the transformed cells are available for preparation for the insertion of foreign genes into higher plants. The vect rs comprise, for example, pBR322, pUC series, M13mp series, pACYC184, etc.

Accordingly, the sequence encoding the *B.t.* toxin can be inserted into the vector at a suitable restriction site. The resulting plasmid is used for transformation into *E. coli*. The *E. coli* cells are cultivated in a suitable nutrient medium, then harvested and lysed. The plasmid is recovered. Sequence analysis, restriction analysis, electrophoresis, and other biochemical-molecular biological methods are generally carried out as methods of analysis. After each manipulation, the DNA sequence used can be cleaved and joined to the next DNA sequence. Each plasmid sequence can be cloned in the same or other plasmids. Depending on the method of inserting desired genes into the plant, other DNA sequences may be necessary. If, for example, the Ti or Ri plasmid is used for the transformation of the plant cell, then at least the right border, but often the right and the left border of the Ti or Ri plasmid T-DNA, has to be joined as the flanking region of the genes to be inserted.

The use of T-DNA for the transformation of plant cells has been intensively researched and sufficiently described in EP 0 120 516; Hoekema (1985) In: *The Binary Plant Vector System*, Offset-durkkerij Kanters B.V., Alblasserdam, Chapter 5; Fraley *et al.*, *Crit. Rev. Plant Sci.* 4:1-46; and An *et al.* (1985) *EMBO J.* 4:277-287.

Once the inserted DNA has been integrated in the genome, it is relatively stable there and, as a rule, does not come out again. It normally contains a selection marker that confers on the transformed plant cells resistance to a biocide or an antibiotic, such as kanamycin, G 418, bleomycin, hygromycin, or chloramphenicol, *inter alia*. The individually employed marker should accordingly permit the selection of transformed cells rather than cells that do not contain the inserted DNA.

A large number of techniques are available for inserting DNA into a plant host cell. Those techniques include transformation with T-DNA using *Agrobacterium tumefaciens* or *Agrobacterium rhizogenes* as transformation agent, fusion, injection, or electroporation as well as other possible methods. If agrobacteria are used for the transformation, the DNA to be inserted has to be cloned into special plasmids, namely either into an intermediate vector or into a binary vector. The intermediate vectors can be integrated into the Ti or Ri plasmid by homologous recombination owing to sequences that are homologous to sequences in the T-DNA. The Ti or Ri plasmid also comprises the *vir* region necessary for the transfer of the T-DNA. Intermediate vectors cannot replicate themselves in agrobacteria. The intermediate vector can be transferred into *Agrobacterium tumefaciens* by means of a helper plasmid (conjugation). Binary vectors can replicate themselves both in *E. coli* and in agrobacteria. They comprise a selection marker gene and a linker or polylinker which are framed by the right and left T-DNA border regions. They can be transformed directly into agrobacteria (Holsters *et al.* [1978] *Mol. Gen. Genet.* 163:181-187). The agrobacterium used as host cell is to comprise a plasmid carrying a *vir* region. The *vir* region is necessary for the transfer of the T-DNA into the plant cell. Additional T-DNA may be contained. The bacterium so transformed is used for the transformation of plant cells. Plant

explants can advantageously be cultivated with *Agrobacterium tumefaciens* or *Agrobacterium rhizogenes* for the transfer of the DNA into the plant cell. Whole plants can then be regenerated from the infected plant material (for example, pieces of leaf, segments of stalk, roots, but also protoplasts or suspension-cultivated cells) in a suitable medium, which may contain antibiotics or biocides for selection. The plants so obtained can then be tested for the presence of the inserted DNA. No special demands are made of the plasmids in the case of injection and electroporation. It is possible to use ordinary plasmids, such as, for example, pUC derivatives.

The transformed cells grow inside the plants in the usual manner. They can form germ cells and transmit the transformed trait(s) to progeny plants. Such plants can be grown in the normal manner and crossed with plants that have the same transformed hereditary factors or other hereditary factors. The resulting hybrid individuals have the corresponding phenotypic properties.

Example 6 – Cloning of Novel *B.t.* Genes Into Insect Viruses

A number of viruses are known to infect insects. These viruses include, for example, baculoviruses and entomopoxviruses. In one embodiment of the subject invention, lepidopteran-active genes, as described herein, can be placed with the genome of the insect virus, thus enhancing the pathogenicity of the virus. Methods for constructing insect viruses which comprise *B.t.* toxin genes are well known and readily practiced by those skilled in the art. These procedures are described, for example, in Merryweather *et al.* (Merryweather, A.T., U. Weyer, M.P.G. Harris, M. Hirst, T. Booth, R.D. Possee [1990] *J. Gen. Virol. 71*:1535-1544) and Martens *et al.* (Martens, J.W.M., G. Honee, D. Zuidema, J.W.M. van Lent, B. Visser, J.M. Vlak [1990] *Appl. Environmental Microbiol. 56*(9):2764-2770).

It should be understood that the examples and embodiments described herein are for illustrative purposes only and that various modifications or changes in light thereof will be suggested to persons skilled in the art and are to be included within the spirit and purview of this application and the scope of the appended claims.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT NAME(S): MYCOGEN CORPORATION
STREET ADDRESS: 5501 Oberlin Drive
CITY: San Diego
STATE/PROVINCE: California
COUNTRY: US
POSTAL CODE/ZIP: 92121
PHONE NUMBER: (619) 453-8030
FAX NUMBER: (619) 453-6991

(ii) TITLE OF INVENTION: Protein Toxins Active Against Lepidopteran Pests

(iii) NUMBER OF SEQUENCES: 27

(iv) CORRESPONDENCE ADDRESS:
(A) ADDRESSEE: Saliwanchik & Saliwanchik
(B) STREET: 2421 N.W. 41st Street, Suite A-1
(C) CITY: Gainesville
(D) STATE: FL
(E) COUNTRY: US
(F) ZIP: 32606

(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.25

(vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER:
(B) FILING DATE:
(C) CLASSIFICATION:

(vii) PRIOR APPLICATION DATA:
(A) APPLICATION NUMBER: 07/597,607
(B) FILING DATE: 15-OCT-90
(C) CLASSIFICATION:

(viii) ATTORNEY/AGENT INFORMATION:
(A) NAME: Saliwanchik, David R.
(B) REGISTRATION NUMBER: 31,794
(C) REFERENCE/DOCKET NUMBER: MA50.C1

(ix) TELECOMMUNICATION INFORMATION:
(A) TELEPHONE: (904)375-8100
(B) TELEFAX: (904)372-5800

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 3522 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

(A) ORGANISM: Bacillus thuringiensis
(B) STRAIN: aizawai
(C) INDIVIDUAL ISOLATE: PS81A2

(vii) IMMEDIATE SOURCE:
(A) LIBRARY: Lambdagem - 11 (tm) Library of August Sick
(B) CLONE: 81A2

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

ATGGAGAATA ATATTGAAAA TCAATGCATA CCTTACAATT GTTTAAATAA TCCTGAAGTA 60

GAGATATTAG GGATTGAAAG GTCAAATAGT AACGTAGCAG CAGAAATCGG CTTGGGGCTT 120

AGTCGTCTGC TCGTTTCCCG AATTCCACTA GGGGATTTTA TACTTGGCTT GTTTGATGTA 180

ATATGGGGGG CTATAGGTCC TTCACAATGG GATATATTTT TAGAGCAAAT TGAGCTATTG 240

ATCGGCCAAA GAATAGAGGA ATTCGCTAGG AATCAGGCAA TTTCTAGATT ACAAGGGCTA 300

AGCAATCTTT ACCGAATTTA CACAAATGCT TTTAAAAACT GGGAAGTAGA TCCTACTAAT 360

CCAGCATTAA GAGAAGAGAT GCGTATTCAA TTTAATGACA TGAACAGTGC TCTTACAACA 420

GCTATTCCTC TTTTTTCAGT TCAAGGTTAT GAAATTCCTC TTTTATCAGT ATATGTTCAA 480

GCTGCAAATT TACATTTATC GGTTTTGAGA GATGTTTCAG TGTTTGGACA ACGTTGGGGA 540

TTTGATGTAG CAACAATCAA TAGTCGTTAT AATGATTTAA CTAGGCTTAT TGGCGAATAT 600

ACTGATTATG CTGTACGTTG GTATAATACG GGGTTAAATC GTTTACCACG TAATGAAGGG 660

GTACGAGGAT GGGCAAGATT TAATAGGTTT AGAAGAGAGT TAACAATATC AGTATTAGAT 720

ATTATTTCTT TTTTCCAAAA TTACGATTCT AGATTATATC CAATTCCGAC AATCTATCAA 780

TTAACGCGGG AAGTATATAC AGATCCGGTA ATTAATATAA CTGATTATAG AGTTACCCCA 840

AGTTTCGAGA GTATTGAAAA TTCAGCTATT AGAAGTCCCC ATCTTATGGA TTTCTTAAAT 900

AATATAATTA TTGACACTGA TTTAATTAGA GGCGTTCACT ATTGGGCGGG GCATCGTGTA 960

ACTTCTCATT TTACCGGTAG TTCGCAAGTG ATAAGCTCCC CTCAATACGG GATAACTGCA 1020

AACGCAGAAC CGAGTCGAAC TATTGCTCCT AGCACTTTTC CAGGTCTTAA TCTATTTTAT 1080

AGAACACTAT CAGACCCTTT CTTCCGAAGA TCCGATAATA TTATGCCAAC ATTAGGAATA 1140

AATGTAGTGC AGGGGGTAGG ATTCATTCAA CCAAATAATG GTGAAGTTCT ATATAGAAGG 1200

AGAGGAACAG TAGATTCTCT TGATGAGTTG CCAATTGACG GTGAGAATTC ATTAGTTGGA 1260

TATAGTCATA GATTAAGTCA CGTTACATTA ACCAGGTCGT TATATAATAC TAATATAACT 1320

AGCTTGCCAA CATTTGTTTG GACACATCAC AGTGCTACTG ATCGAAATAT AATCTATCCG 1380

GATGTAATTA CACAAATACC ATTGGTAAAA TCATTCTCCC TTACTTCAGG TACCTCTGTA 1440

GTCAGAGGCC CAGGATTTAC AGGAGGGGAT ATCATCCGAA CTAACGTTAA TGGTAATGTA 1500

CTAAGTATGA GTCTTAATTT TAGTAATACA TCATTACAGC GGTATCGCGT GAGAGTTCGT 1560

TATGCTGCTT CTCAAACAAT GGTCATGAGA GTAAATGTTG GAGGGAGTAC TACTTTTGAT 1620

CAAGGATTCC CTAGTACTAT GAGTGCAAAT GGGTCTTTGA CATCTCAATC ATTTAGATTT 1680

GCAGAATTTC CTGTAGGCAT TAGTACATCT GGCAGTCAAA CTGCTGGAAT AAGTATAAGT 1740

AATAATCCAG GTAGACAAAC GTTTCACTTA GATAGAATTG AATTTATCCC AGTTGATGCA 1800

ACATTTGAAG CAGAATATGA TTTAGAAAGA GCACAAAAGG CGGTGAATTC GCTGTTTACT 1860

TCTTCCAATC AAATCGAGTT AAAAACAGAT GTGACGGATT ATCATATTGA TCAAGTATCC 1920

AATTTAGTAG ATTGTTTATC CGATGAATTT TGTCTGGATG AAAAGCGAGA ATTGTCCGAG 1980

AAAGTCAAAC ATGCGAAGCG ACTCAGTGAT GAGCGGAATT TACTTCAAGA TCCAAACTTC 2040

AGAGGGATCA ATAGGCAACC AGACCGTGGC TGGAGAGGAA GTACGGATAT TACCATCCAA 2100

GGAGGAGATG ACGTATTCAA AGAGAATTAC GTCACACTAC CAGGTACCTT TGATGAGTGC 2160

TATCCAACGT ATTTGTATCA AAAAATAGAT GAGTCGAAAT TAAAAGCCTA TAACCGTTAC 2220

CAATTAAGAG GGTATATCGA AGATAGTCAA GACTTAGAAA TCTATTTAAT TCGCTACAAT 2280

GCAAAACACG AAACAGTAAA TGTACCAGGT ACGGGTTCCT TATGGCCGCT TTCAGTCGAA 2340

AGTCCAATTG GAAGGTGTGG AGAACCGAAT CGGTGTGTGC CACACCTTGA ATGGAATCCT 2400

GATTTAGATT GTTCCTGCAG AGACGGGGAA AAATGTGCAC ATCATTCCCA TCATTTCTCC 2460

TTGGACATTG ATGTTGGATG CACAGACTTG CAAGAGGATC TAGGCGTGTG GGTTGTATTC 2520

AAGATTAAGA CGCAGGAAGG TTATGCAAGA TTAGGAAATC TGGAATTTAT CGAAGAGAAA 2580

CCATTAATTG GAGAAGCACT GTCTCGTGTG AAGAGAGCGG AAAAAAAATG GAGAGACAAA 2640

CGGGAAAAAC TACAATTGGA AACAAAACGA GTATATACAG AGGCAAAAGA AGCTGTGGAT 2700

GCTTTATTCG TAGATTCTCA ATATGATAGA TTACAAGCAG ATACAAACAT TGGTATGATT 2760

CATGCGGCAG ATAGACTTGT TCATCAGATC CACGAGGCTT ATCTTCCAGA ACTACCTTTC 2820

ATTCCAGGAA TAAATGTGGT GATTTTTGAA GAATTAGAAA ACCGTATTTC TACTGCATTA 2880

TCCCTATATG ATGCGAGAAA TGTCATTAAA AATGGCGATT TCAATAATGG CTTATCATGC 2940

TGGAACGTGA AAGGGCATGT AGATGTAGTA GAACAAAACA ACCACCGTTC GGTCCTTGTT 3000

GTCCCGGAAT GGGAAGCAGA AGTGTCACAA ACAATTCGTG TCTGTCCGGG GCGTGGCTAT 3060

ATCCTCCGTG TTACAGCGTA CAAAGAGGGA TATGGAGAAG GTTGCGTAAC CATCCATGAG 3120

ATCGAGAACA ATACAGACGA ACTAAAATTT AAAAACTGTG AAGAAGAGGA AGTGTATCCA 3180

ACGGATACAG GAACGTGTAA TGATTATACT GCACACCAAG GTACAGCAGG ATCCACAGAT 3240

TCATGTAATT CCCGTAATAT CAGATATGAG GATGCATATG AAATGAATAC TACAGCATCT 3300

GTTAATTACA AACCGACTTA CGAAGAAGAA AGGTATACAG ATGTACAAGG AGATAATCAT 3360

TGTGAATATG ACAGAGGGTA TGTGAATTAT CGACCAGTAC CAGCTGGTTA TGTGACAAAA 3420

GAATTAGAGT ACTTCCCAGA AACCGATAAG GTATGGATTG AGATCGGAGA AACGGAAGGG 3480

AAGTTTATTG TAGACAATGT CGAATTACTC CTTATGGAGG AA 3522

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1174 amino acids

(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(iii) HYPOTHETICAL: YES

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Bacillus thuringiensis
(B) STRAIN: aizawai
(C) INDIVIDUAL ISOLATE: PS81A2

(vii) IMMEDIATE SOURCE:
(A) LIBRARY: Lambdagem - 11 (tm) Library of August Sick
(B) CLONE: 81A2

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

Met Glu Asn Asn Ile Glu Asn Gln Cys Ile Pro Tyr Asn Cys Leu Asn
1 5 10 15

Asn Pro Glu Val Glu Ile Leu Gly Ile Glu Arg Ser Asn Ser Asn Val
20 25 30

Ala Ala Glu Ile Gly Leu Gly Leu Ser Arg Leu Leu Val Ser Arg Ile
35 40 45

Pro Leu Gly Asp Phe Ile Leu Gly Leu Phe Asp Val Ile Trp Gly Ala
50 55 60

Ile Gly Pro Ser Gln Trp Asp Ile Phe Leu Glu Gln Ile Glu Leu Leu
65 70 75 80

Ile Gly Gln Arg Ile Glu Glu Phe Ala Arg Asn Gln Ala Ile Ser Arg
85 90 95

Leu Gln Gly Leu Ser Asn Leu Tyr Arg Ile Tyr Thr Asn Ala Phe Lys
100 105 110

Asn Trp Glu Val Asp Pro Thr Asn Pro Ala Leu Arg Glu Glu Met Arg
115 120 125

Ile Gln Phe Asn Asp Met Asn Ser Ala Leu Thr Thr Ala Ile Pro Leu
130 135 140

Phe Ser Val Gln Gly Tyr Glu Ile Pro Leu Leu Ser Val Tyr Val Gln
145 150 155 160

Ala Ala Asn Leu His Leu Ser Val Leu Arg Asp Val Ser Val Phe Gly
165 170 175

Gln Arg Trp Gly Phe Asp Val Ala Thr Ile Asn Ser Arg Tyr Asn Asp
180 185 190

Leu Thr Arg Leu Ile Gly Glu Tyr Thr Asp Tyr Ala Val Arg Trp Tyr
195 200 205

Asn Thr Gly Leu Asn Arg Leu Pro Arg Asn Glu Gly Val Arg Gly Trp
210 215 220

Ala Arg Phe Asn Arg Phe Arg Arg Glu Leu Thr Ile S r Val Leu Asp
225 230 235 240

Ile Ile Ser Phe Phe Gln Asn Tyr Asp S r Arg Leu Tyr Pro Ile Pro
245 250 255

Thr Ile Tyr Gln Leu Thr Arg Glu Val Tyr Thr Asp Pro Val Ile Asn
260 265 270

Ile Thr Asp Tyr Arg Val Thr Pro Ser Phe Glu Ser Ile Glu Asn Ser
275 280 285

Ala Ile Arg Ser Pro His Leu Met Asp Phe Leu Asn Asn Ile Ile Ile
290 295 300

Asp Thr Asp Leu Ile Arg Gly Val His Tyr Trp Ala Gly His Arg Val
305 310 315 320

Thr Ser His Phe Thr Gly Ser Ser Gln Val Ile Ser Ser Pro Gln Tyr
325 330 335

Gly Ile Thr Ala Asn Ala Glu Pro Ser Arg Thr Ile Ala Pro Ser Thr
340 345 350

Phe Pro Gly Leu Asn Leu Phe Tyr Arg Thr Leu Ser Asp Pro Phe Phe
355 360 365

Arg Arg Ser Asp Asn Ile Met Pro Thr Leu Gly Ile Asn Val Val Gln
370 375 380

Gly Val Gly Phe Ile Gln Pro Asn Asn Gly Glu Val Leu Tyr Arg Arg
385 390 395 400

Arg Gly Thr Val Asp Ser Leu Asp Glu Leu Pro Ile Asp Gly Glu Asn
405 410 415

Ser Leu Val Gly Tyr Ser His Arg Leu Ser His Val Thr Leu Thr Arg
420 425 430

Ser Leu Tyr Asn Thr Asn Ile Thr Ser Leu Pro Thr Phe Val Trp Thr
435 440 445

His His Ser Ala Thr Asp Arg Asn Ile Ile Tyr Pro Asp Val Ile Thr
450 455 460

Gln Ile Pro Leu Val Lys Ser Phe Ser Leu Thr Ser Gly Thr Ser Val
465 470 475 480

Val Arg Gly Pro Gly Phe Thr Gly Gly Asp Ile Ile Arg Thr Asn Val
485 490 495

Asn Gly Asn Val Leu Ser Met Ser Leu Asn Phe Ser Asn Thr Ser Leu
500 505 510

Gln Arg Tyr Arg Val Arg Val Arg Tyr Ala Ala Ser Gln Thr Met Val
515 520 525

Met Arg Val Asn Val Gly Gly Ser Thr Thr Phe Asp Gln Gly Phe Pro
530 535 540

Ser Thr Met Ser Ala Asn Gly Ser Leu Thr Ser Gln Ser Phe Arg Phe
545 550 555 560

Ala Glu Phe Pro Val Gly Il Ser Thr Ser Gly Ser Gln Thr Ala Gly
565 570 575

Ile S r Ile Ser Asn Asn Pr Gly Arg Gln Thr Phe His Leu Asp Arg
580 585 590

Ile Glu Phe Ile Pro Val Asp Ala Thr Phe Glu Ala Glu Tyr Asp Leu
595 600 605

Glu Arg Ala Gln Lys Ala Val Asn Ser Leu Phe Thr Ser Ser Asn Gln
610 615 620

Ile Glu Leu Lys Thr Asp Val Thr Asp Tyr His Ile Asp Gln Val Ser
625 630 635 640

Asn Leu Val Asp Cys Leu Ser Asp Glu Phe Cys Leu Asp Glu Lys Arg
645 650 655

Glu Leu Ser Glu Lys Val Lys His Ala Lys Arg Leu Ser Asp Glu Arg
660 665 670

Asn Leu Leu Gln Asp Pro Asn Phe Arg Gly Ile Asn Arg Gln Pro Asp
675 680 685

Arg Gly Trp Arg Gly Ser Thr Asp Ile Thr Ile Gln Gly Gly Asp Asp
690 695 700

Val Phe Lys Glu Asn Tyr Val Thr Leu Pro Gly Thr Phe Asp Glu Cys
705 710 715 720

Tyr Pro Thr Tyr Leu Tyr Gln Lys Ile Asp Glu Ser Lys Leu Lys Ala
725 730 735

Tyr Asn Arg Tyr Gln Leu Arg Gly Tyr Ile Glu Asp Ser Gln Asp Leu
740 745 750

Glu Ile Tyr Leu Ile Arg Tyr Asn Ala Lys His Glu Thr Val Asn Val
755 760 765

Pro Gly Thr Gly Ser Leu Trp Pro Leu Ser Val Glu Ser Pro Ile Gly
770 775 780

Arg Cys Gly Glu Pro Asn Arg Cys Val Pro His Leu Glu Trp Asn Pro
785 790 795 800

Asp Leu Asp Cys Ser Cys Arg Asp Gly Glu Lys Cys Ala His His Ser
805 810 815

His His Phe Ser Leu Asp Ile Asp Val Gly Cys Thr Asp Leu Gln Glu
820 825 830

Asp Leu Gly Val Trp Val Val Phe Lys Ile Lys Thr Gln Glu Gly Tyr
835 840 845

Ala Arg Leu Gly Asn Leu Glu Phe Ile Glu Glu Lys Pro Leu Ile Gly
850 855 860

Glu Ala Leu Ser Arg Val Lys Arg Ala Glu Lys Lys Trp Arg Asp Lys
865 870 875 880

Arg Glu Lys Leu Gln Leu Glu Thr Lys Arg Val Tyr Thr Glu Ala Lys
885 890 895

Glu Ala Val Asp Ala Leu Phe Val Asp Ser Gln Tyr Asp Arg Leu Gln
900 905 910

Ala Asp Thr Asn Ile Gly Met Ile His Ala Ala Asp Arg Leu Val His
915 920 925

Gln Il His Glu Ala Tyr Leu Pro Glu Leu Pro Phe Ile Pro Gly Ile
930 935 940

```
Asn Val Val Ile Phe Glu Glu Leu Glu Asn Arg Ile S r Thr Ala Leu
945                 950                 955                 960

Ser Leu Tyr Asp Ala Arg Asn Val Ile Lys Asn Gly Asp Phe Asn Asn
                965                 970                 975

Gly Leu Ser Cys Trp Asn Val Lys Gly His Val Asp Val Val Glu Gln
            980                 985                 990

Asn Asn His Arg Ser Val Leu Val Val Pro Glu Trp Glu Ala Glu Val
        995                 1000                1005

Ser Gln Thr Ile Arg Val Cys Pro Gly Arg Gly Tyr Ile Leu Arg Val
    1010                1015                1020

Thr Ala Tyr Lys Glu Gly Tyr Gly Glu Gly Cys Val Thr Ile His Glu
1025                1030                1035                1040

Ile Glu Asn Asn Thr Asp Glu Leu Lys Phe Lys Asn Cys Glu Glu Glu
                1045                1050                1055

Glu Val Tyr Pro Thr Asp Thr Gly Thr Cys Asn Asp Tyr Thr Ala His
            1060                1065                1070

Gln Gly Thr Ala Gly Ser Thr Asp Ser Cys Asn Ser Arg Asn Ile Arg
        1075                1080                1085

Tyr Glu Asp Ala Tyr Glu Met Asn Thr Thr Ala Ser Val Asn Tyr Lys
    1090                1095                1100

Pro Thr Tyr Glu Glu Glu Arg Tyr Thr Asp Val Gln Gly Asp Asn His
1105                1110                1115                1120

Cys Glu Tyr Asp Arg Gly Tyr Val Asn Tyr Arg Pro Val Pro Ala Gly
                1125                1130                1135

Tyr Val Thr Lys Glu Leu Glu Tyr Phe Pro Glu Thr Asp Lys Val Trp
            1140                1145                1150

Ile Glu Ile Gly Glu Thr Glu Gly Lys Phe Ile Val Asp Asn Val Glu
        1155                1160                1165

Leu Leu Leu Met Glu Glu
    1170
```

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 3504 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Bacillus thuringiensis
(B) STRAIN: Morrissoni
(C) INDIVIDUAL ISOLATE: PS91C2

(vii) IMMEDIATE SOURCE:
(A) LIBRARY: LambdaGem (TM) - 11 Library f Ter sa

Thompson
(B) CLONE: 91C2

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

ATGAAGAATA ACATTCAAAA TCAATGCGTA CCTTACAATT GTTTAAGTAA TCCTGAAGTA 60
GAAATATTAA GTGAAGAAAG AAGTACTGGC AGATTACCGT TAGATATATC CTTGTCGCTT 120
ACACGTTTCC TTTTGAGTGA ATTTGTTCCA GGTGTGGGAG TTGCGTTTGG ATTATTTGAT 180
TTAATATGGG GTTTTATAAC TCCTTCTGAA TGGAGTTTAT TTCTTTTACA GATTGAACAA 240
CTGATTGAAC AAAGAATTGA AACATTGGAA AGGAACCGGG CAATTACTAC ATTACGAGGG 300
TTAGCGGATA GCTATGAAGT TTACCTTGAG GCACTAAGAG AGTGGGAAGA AAATCCTAAT 360
AATGCACAAT TAAGGGAAGA TGTGCGTATT CGATTTGCTA ATACAGACGA CGCTTTAATA 420
ACAGCAATAA ATAATTTTAC ACTTACAAGT TTTGAAATCC CTCTTTTATC GGTCTATGTT 480
CAAGCGGCGA ATCTACATTT ATCACTATTA AGAGATGCTG TATCGTTTGG GCAGGGTTGG 540
GGGCTGGATA TAGCTACTGT TAATAATCAT TATAATAGAT TAATAAATCT TATTCATAGA 600
TATACGGAAC ATTGTTTGGA CACATACAAT CAAGGATTAG AAAACTTAAG AGGTACTAAT 660
ACTCGACAAT GGTCAAGATT CAATCAGTTT AGGAGAGAGT TAACATTGAC TGTATTAGAT 720
ATCGTTGCTC TTTTTCCGAA CTACGATGCT AGAGCATATC CAATTCAAAC GTCATCCCAA 780
TTAACAAGGG AAATTTATAC AAGTTCAGTA ATTGAAGATT CTCCAGTTTC TGCTAATATA 840
CCTAATGGTT TTAATAGAGC GGAATTTGGA GTTAGACCGC CCCATCTTAT GGACTTTATG 900
AATTCTTTGT TTGTAACTGC AGAGACTGTT AGAAGTCAAA CTGTGTGGGG AGGACACTTA 960
GTTAGTTCAC GAAATACGGC TGGTAACCCT ATAAATTTCC CTATTTATGG GGTCTTCAAT 1020
CCTGGTGGCG CCATTTGGAT TGCAGATGAG GATCCACGTC CTTTTTATCG GACATTATCA 1080
GATCCTGTTT TTGTCCGAGG AGGATTTGGG GATCCTCATT ATGTACTTGG GCTTAGGGGA 1140
GTAGGATTTC AACAAACTGG TACGAACCAC ACCCGAACAT TTAGAAATAG TGGGACCATA 1200
GATTCTCTAG ATGAAATCCC ACCTCAGGAT AATAGTGGGG CACCTTGGAA TGATTATAGT 1260
CATGTATTAA ATCATGTTAC ATTTGTAAGG TGGCCTGGTG AGATTGCAGG AAGTGATTCA 1320
TGGAGAGCGC CAATGTTTTC TTGGACACAC CGTAGTGCAG ATCGTACAAA TATCATTAAT 1380
CCAAATATAA TTACACAAAT ACCTGCTGTA AAAGCACACA ATCTTCATTC GGGTTCTACG 1440
GTTGTTAGAG GACCCGGGTT TACAGGTGGT GATCTCTTAC GAAGAACGAA TACTGGTACA 1500
TTTGCAGATA TAAGAGTAAA TATTACTGGG CCATTATCTC AAAGATATCG TGTAAGAATT 1560
CGCTATGCTT CTACGACAGA TTTACAATTT TTCACGAGAA TCAATGGAAC TTCTGTAAAT 1620
CAAGGTAATT TCCAAAGAAC TATGAATAGA GGGGATAATT TAGAATCTGG AAACTTTAGG 1680
ACTGCAGGAT TTAGTACGCC TTTTAGTTTT TCAAATGCGC AAAGTACATT CACATTGGGT 1740
ACTCAGGCTT TTTCAAATCA GGAAGTTTAT ATAGATCGAA TTGAATTTGT CCCGGCAGAA 1800
GTAACATTCG AGGCAGAATC TGATTTAGAA AGAGCGCAAA AGGCGGTGAA TGCCCTGTTT 1860

ACTTCTACAA GCCAACTAGG GCTAAAAACA AATGTAACGG GTTACCATAT TGATCAAGTG 1920

TCCAATTTAG TTGCGTGTTT ATCGGATGAA TTTTGTCTGG ATGAAAAGAG AGAATTGTCC 1980

GAGAAAGTTA AACATGCGAA GCGACTCAGT GATAAGCGGA ATTTACTTCA AGATCCAAAC 2040

TTCAGAGGGA TCAATAGGCA ACCAGACCAT GGCTGGAGAG GAAGTACGGA TATTACTATC 2100

CAAGGAGGAG ATGACGTATT CAAAGAGAAT TACGTTACGC TACCGGGTAC TTTTGATGAG 2160

TGCTATCCAA CGTATTTATA TCAAAAAATA GATGAGTCGA AATTAAAAGC CTATACCCGT 2220

TATCAATTAA GAGGGTATAT CGAAGATAGT CAAGACTTAG AAATCTATTT AATTCGTTAC 2280

AATTCAAAAC ACGAAATAGT AAATGTACCA GGTACAGGGA GTTTATGGCC TCTTTCTGTA 2340

GAAAATCAAA TTGGACCTTG TGGAGAACCG AATCGATGCG CGCCACACCT TGAATGGAAT 2400

CCTGATTTAC ACTGTTCCTG CAGAGACGGG GAAAAATGTG TGCATCATTC TCATCATTTC 2460

TCTTTGGACA TTGATGTCGG ATGTACAGAT TTAAATGAGG ACCTAGGTGT ATGGTTGATA 2520

TTCAAGATTA AGACGCAAGA TGGCCACGCA AGACTAGGGA ATCTAGAGTT TCTCGAAGAG 2580

GAACCGTTAT TAGGCGAAGC GTTAGGACGT GTGAAGAGAG CGGAGAAGAA GTGGAGAGAC 2640

AAACGCGAGA AACTGCAGTT GGAAACAAAT ATTGTCTATA AAGAGGCAAA AGAATCTGTA 2700

GATGCTTTAT TTGTAAACTC TCAATATGAT AGATTACAAG CGGATACGAA CATCGCGATG 2760

ATTCATGCGG CAGATAAACG CGTTCATAGA ATCCGGGAAG CGTATCTGCC AGAGTTGTCT 2820

GTGATTCCAG GTGTCAATGC GGCCATTTTC GAAGAATTAG AGGGACGTAT TTTTACAGCG 2880

TATTCCTTAT ATGATGCGAG AAATGTTATT AAAAATGGCA ATTTCAATAA TGGCTTATTA 2940

TGCTGGAACG TGAAAGGGCA TGTAGATGTA GAAGAGCAAA ACAACCACCG TTCGGTCCTT 3000

GTTGTTCCGG AATGGGAAGC AGAAGTGTCA CAAGAAGTTC GTGTCTGTCC GGGTCGTGGC 3060

TATATCCTTC GTGTCACAGC GTACAAAGAG GGATATGGAG AAGGCTGCGT AACTATTCAT 3120

GAAGTCGATA ATAATACAGA CGAATTGAAG TTTAGCAACT GTGAGAAAGA ACAAGTATAT 3180

CCAGGTAATA CGGTAGCATG TAATGATTAT AATAAGAATC ACGGTGCGAA TGCATGTAGT 3240

TCTCGTAATC GTGGATATGA CGAATCTTAT GAAAGTAATT CTTCCATACC AGCTGATTAT 3300

GCACCGGTTT ATGAAGAAGA AGCGTATACA GATGGACAAA GAGGGAATCC TTGTGAATTT 3360

AACAGAGGGC ATACACCATT ACCAGCTGGT TATGTGACAG CAGAGTTAGA GTACTTCCCA 3420

GAAACGGATA CAGTATGGGT TGAGATTGGA GAAACGGAAG GAACATTTAT CGTGGACAGT 3480

GTGGAATTAC TCCTTATGGA GGAA 3504

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1168 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(iii) HYPOTHETICAL: YES

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: BACILLUS THURINGIENSIS
(B) STRAIN: Morrissoni
(C) INDIVIDUAL ISOLATE: PS91C2

(vii) IMMEDIATE SOURCE:
(A) LIBRARY: LAMBDAGEM (TM) - 11 LIBRARY OF TERESA THOMPSON
(B) CLONE: 91C2

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

Met Lys Asn Asn Ile Gln Asn Gln Cys Val Pro Tyr Asn Cys Leu Ser
1 5 10 15

Asn Pro Glu Val Glu Ile Leu Ser Glu Glu Arg Ser Thr Gly Arg Leu
20 25 30

Pro Leu Asp Ile Ser Leu Ser Leu Thr Arg Phe Leu Leu Ser Glu Phe
35 40 45

Val Pro Gly Val Gly Val Ala Phe Gly Leu Phe Asp Leu Ile Trp Gly
50 55 60

Phe Ile Thr Pro Ser Glu Trp Ser Leu Phe Leu Leu Gln Ile Glu Gln
65 70 75 80

Leu Ile Glu Gln Arg Ile Glu Thr Leu Glu Arg Asn Arg Ala Ile Thr
85 90 95

Thr Leu Arg Gly Leu Ala Asp Ser Tyr Glu Val Tyr Leu Glu Ala Leu
100 105 110

Arg Glu Trp Glu Glu Asn Pro Asn Asn Ala Gln Leu Arg Glu Asp Val
115 120 125

Arg Ile Arg Phe Ala Asn Thr Asp Asp Ala Leu Ile Thr Ala Ile Asn
130 135 140

Asn Phe Thr Leu Thr Ser Phe Glu Ile Pro Leu Leu Ser Val Tyr Val
145 150 155 160

Gln Ala Ala Asn Leu His Leu Ser Leu Leu Arg Asp Ala Val Ser Phe
165 170 175

Gly Gln Gly Trp Gly Leu Asp Ile Ala Thr Val Asn Asn His Tyr Asn
180 185 190

Arg Leu Ile Asn Leu Ile His Arg Tyr Thr Glu His Cys Leu Asp Thr
195 200 205

Tyr Asn Gln Gly Leu Glu Asn Leu Arg Gly Thr Asn Thr Arg Gln Trp
210 215 220

Ser Arg Phe Asn Gln Phe Arg Arg Glu Leu Thr Leu Thr Val Leu Asp
225 230 235 240

Ile Val Ala Leu Phe Pro Asn Tyr Asp Ala Arg Ala Tyr Pro Ile Gln
245 250 255

Thr Ser Ser Gln Leu Thr Arg Glu Ile Tyr Thr S r Ser Val Ile Glu
260 265 270

Asp Ser Pro Val Ser Ala Asn Ile Pro Asn Gly Phe Asn Arg Ala Glu
275 280 285

Phe Gly Val Arg Pro Pro His Leu Met Asp Phe Met Asn Ser Leu Phe
290 295 300

Val Thr Ala Glu Thr Val Arg Ser Gln Thr Val Trp Gly Gly His Leu
305 310 315 320

Val Ser Ser Arg Asn Thr Ala Gly Asn Pro Ile Asn Phe Pro Ile Tyr
325 330 335

Gly Val Phe Asn Pro Gly Gly Ala Ile Trp Ile Ala Asp Glu Asp Pro
340 345 350

Arg Pro Phe Tyr Arg Thr Leu Ser Asp Pro Val Phe Val Arg Gly Gly
355 360 365

Phe Gly Asp Pro His Tyr Val Leu Gly Leu Arg Gly Val Gly Phe Gln
370 375 380

Gln Thr Gly Thr Asn His Thr Arg Thr Phe Arg Asn Ser Gly Thr Ile
385 390 395 400

Asp Ser Leu Asp Glu Ile Pro Pro Gln Asp Asn Ser Gly Ala Pro Trp
405 410 415

Asn Asp Tyr Ser His Val Leu Asn His Val Thr Phe Val Arg Trp Pro
420 425 430

Gly Glu Ile Ala Gly Ser Asp Ser Trp Arg Ala Pro Met Phe Ser Trp
435 440 445

Thr His Arg Ser Ala Asp Arg Thr Asn Ile Ile Asn Pro Asn Ile Ile
450 455 460

Thr Gln Ile Pro Ala Val Lys Ala His Asn Leu His Ser Gly Ser Thr
465 470 475 480

Val Val Arg Gly Pro Gly Phe Thr Gly Gly Asp Leu Leu Arg Arg Thr
485 490 495

Asn Thr Gly Thr Phe Ala Asp Ile Arg Val Asn Ile Thr Gly Pro Leu
500 505 510

Ser Gln Arg Tyr Arg Val Arg Ile Arg Tyr Ala Ser Thr Thr Asp Leu
515 520 525

Gln Phe Phe Thr Arg Ile Asn Gly Thr Ser Val Asn Gln Gly Asn Phe
530 535 540

Gln Arg Thr Met Asn Arg Gly Asp Asn Leu Glu Ser Gly Asn Phe Arg
545 550 555 560

Thr Ala Gly Phe Ser Thr Pro Phe Ser Phe Ser Asn Ala Gln Ser Thr
565 570 575

Phe Thr Leu Gly Thr Gln Ala Phe Ser Asn Gln Glu Val Tyr Ile Asp
580 585 590

Arg Ile Glu Phe Val Pro Ala Glu Val Thr Phe Glu Ala Glu Ser Asp
595 600 605

Leu Glu Arg Ala Gln Lys Ala Val Asn Ala Leu Phe Thr Ser Thr Ser
610 615 620

Gln Leu Gly Leu Lys Thr Asn Val Thr Gly Tyr His Ile Asp Gln Val
625 630 635 640

Ser Asn Leu Val Ala Cys Leu Ser Asp Glu Phe Cys Leu Asp Glu Lys
645 650 655

Arg Glu Leu Ser Glu Lys Val Lys His Ala Lys Arg Leu Ser Asp Lys
660 665 670

Arg Asn Leu Leu Gln Asp Pro Asn Phe Arg Gly Ile Asn Arg Gln Pro
675 680 685

Asp His Gly Trp Arg Gly Ser Thr Asp Ile Thr Ile Gln Gly Gly Asp
690 695 700

Asp Val Phe Lys Glu Asn Tyr Val Thr Leu Pro Gly Thr Phe Asp Glu
705 710 715 720

Cys Tyr Pro Thr Tyr Leu Tyr Gln Lys Ile Asp Glu Ser Lys Leu Lys
725 730 735

Ala Tyr Thr Arg Tyr Gln Leu Arg Gly Tyr Ile Glu Asp Ser Gln Asp
740 745 750

Leu Glu Ile Tyr Leu Ile Arg Tyr Asn Ser Lys His Glu Ile Val Asn
755 760 765

Val Pro Gly Thr Gly Ser Leu Trp Pro Leu Ser Val Glu Asn Gln Ile
770 775 780

Gly Pro Cys Gly Glu Pro Asn Arg Cys Ala Pro His Leu Glu Trp Asn
785 790 795 800

Pro Asp Leu His Cys Ser Cys Arg Asp Gly Glu Lys Cys Val His His
805 810 815

Ser His His Phe Ser Leu Asp Ile Asp Val Gly Cys Thr Asp Leu Asn
820 825 830

Glu Asp Leu Gly Val Trp Leu Ile Phe Lys Ile Lys Thr Gln Asp Gly
835 840 845

His Ala Arg Leu Gly Asn Leu Glu Phe Leu Glu Glu Glu Pro Leu Leu
850 855 860

Gly Glu Ala Leu Gly Arg Val Lys Arg Ala Glu Lys Lys Trp Arg Asp
865 870 875 880

Lys Arg Glu Lys Leu Gln Leu Glu Thr Asn Ile Val Tyr Lys Glu Ala
885 890 895

Lys Glu Ser Val Asp Ala Leu Phe Val Asn Ser Gln Tyr Asp Arg Leu
900 905 910

Gln Ala Asp Thr Asn Ile Ala Met Ile His Ala Ala Asp Lys Arg Val
915 920 925

His Arg Ile Arg Glu Ala Tyr Leu Pro Glu Leu Ser Val Ile Pro Gly
930 935 940

Val Asn Ala Ala Ile Phe Glu Glu Leu Glu Gly Arg Ile Phe Thr Ala
945 950 955 960

Tyr Ser Leu Tyr Asp Ala Arg Asn Val Ile Lys Asn Gly Asn Phe Asn
965 970 975

Asn Gly Leu Leu Cys Trp Asn Val Lys Gly His Val Asp Val Glu Glu
980 985 990

Gln Asn Asn His Arg Ser Val Leu Val Val Pro Glu Trp Glu Ala Glu
995 1000 1005

Val Ser Gln Glu Val Arg Val Cys Pro Gly Arg Gly Tyr Ile Leu Arg
1010 1015 1020

Val Thr Ala Tyr Lys Glu Gly Tyr Gly Glu Gly Cys Val Thr Ile His
1025 1030 1035 1040

Glu Val Asp Asn Asn Thr Asp Glu Leu Lys Phe Ser Asn Cys Glu Lys
1045 1050 1055

Glu Gln Val Tyr Pro Gly Asn Thr Val Ala Cys Asn Asp Tyr Asn Lys
1060 1065 1070

Asn His Gly Ala Asn Ala Cys Ser Ser Arg Asn Arg Gly Tyr Asp Glu
1075 1080 1085

Ser Tyr Glu Ser Asn Ser Ser Ile Pro Ala Asp Tyr Ala Pro Val Tyr
1090 1095 1100

Glu Glu Glu Ala Tyr Thr Asp Gly Gln Arg Gly Asn Pro Cys Glu Phe
1105 1110 1115 1120

Asn Arg Gly His Thr Pro Leu Pro Ala Gly Tyr Val Thr Ala Glu Leu
1125 1130 1135

Glu Tyr Phe Pro Glu Thr Asp Thr Val Trp Val Glu Ile Gly Glu Thr
1140 1145 1150

Glu Gly Thr Phe Ile Val Asp Ser Val Glu Leu Leu Leu Met Glu Glu
1155 1160 1165

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 19 bases
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (synthetic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

ATGATTCATG CGGCAGATA 19

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 36 bases
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (synthetic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

GAGTGGGAAG CAGATCTTAA TAATGCACAA TTAAGG 36

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 25 bases
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (synthetic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

ATACYCGATC GATATGATAR TCCGT 25

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 23 bases
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (synthetic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

CCCAATGTGA ATGTACTTTG CGC 23

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 8 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

Ser Thr Gly Arg Leu Pro Leu Asp
5

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24 bases
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (synthetic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

AGTACWGGMA GRTTACCRTT RGAY 24

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 8 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

Glu Asp Ser Pro Val Ser Ala Asn
5

(2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24 bases
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (synthetic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

GARGATTCWC CAGTWTCWGC WAAT 24

(2) INFORMATION FOR SEQ ID NO:13:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 10 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

Asn Gly Phe Asn Arg Ala Glu Phe Gly Val
5 10

(2) INFORMATION FOR SEQ ID NO:14:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 31 bases
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (synthetic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

AATGGWTTTA ATAGTGCTGA ATTTGGGAGT W 31

(2) INFORMATION FOR SEQ ID NO:15:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 10 amino acids
(B) TYPE: amino acid

(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

Val Thr Ala Glu Thr Val Arg Ser Gln Thr
5 10

(2) INFORMATION FOR SEQ ID NO:16:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 30 bases
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (synthetic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

GTAACWGCAG ARACWGTWAG WAGTCAAACW 30

(2) INFORMATION FOR SEQ ID NO:17:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 13 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

Val Phe Asn Pro Gly Gly Ala Ile Trp Ile Ala Asp Glu
5 10

(2) INFORMATION FOR SEQ ID NO:18:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 42 bases
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (synthetic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

GTMTTYAATC CWGGWGGMGC MATWTGGATW GCWGATGARG AT 42

(2) INFORMATION FOR SEQ ID NO:19:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 6 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

Val Arg Gly Gly Phe Gly
5

(2) INFORMATION FOR SEQ ID NO:20:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 bases
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (synthetic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:

GTMMGAGGWG GWTTTGGR 18

(2) INFORMATION FOR SEQ ID NO:21:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 7 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:21:

Gly Thr Asn His Thr Arg Thr
5

(2) INFORMATION FOR SEQ ID NO:22:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 bases
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (synthetic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:22:

GGWACRAAYC AYACMMGAAC W 21

(2) INFORMATION FOR SEQ ID NO:23:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 7 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:23:

Val Arg Trp Pro Gly Glu Ile
5

(2) INFORMATION FOR SEQ ID NO:24:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 bases
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (synthetic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:24:

GTWMGATGGC CWGGWGARAT W 21

(2) INFORMATION FOR SEQ ID NO:25:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 6 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:25:

Ser Asp Ser Trp Arg Ala
5

(2) INFORMATION FOR SEQ ID NO:26:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 bases
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (synthetic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:26:

AGTGATTCWT GGAGAGCW 18

(2) INFORMATION FOR SEQ ID NO:27:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1174 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:27:

Met Xaa Asn Asn Ile Gln Asn Gln Cys Val Pro Tyr Asn Cys Leu Xaa
1 5 10 15

Asn Pro Glu Val Glu Ile Leu Xaa Glu Glu Arg Ser Thr Gly Arg Leu
20 25 30

Pro Leu Asp Ile Ser Leu Ser Leu Thr Arg Phe Leu Leu S r Glu Phe
35 40 45

Val Pro Gly Val Gly Val Ala Phe Gly Leu Phe Asp Leu Ile Trp Gly
50 55 60

Phe Ile Thr Pro Ser Xaa Trp Ser Leu Phe Leu Leu Gln Ile Glu Gln
65 70 75 80

Leu Ile Glu Gln Arg Ile Glu Thr Leu Glu Arg Asn Arg Ala Ile Thr
85 90 95

Thr Leu Arg Gly Leu Ala Asp Ser Tyr Glu Xaa Tyr Xaa Glu Ala Leu
100 105 110

Arg Glu Trp Glu Xaa Asn Pro Asn Asn Ala Gln Leu Arg Glu Asp Val
115 120 125

Arg Ile Arg Phe Ala Asn Thr Asp Asp Ala Leu Ile Thr Ala Ile Asn
130 135 140

Asn Phe Thr Leu Thr Ser Phe Glu Ile Pro Leu Leu Ser Val Tyr Val
145 150 155 160

Gln Ala Ala Asn Leu His Leu Ser Leu Leu Arg Asp Ala Val Ser Phe
165 170 175

Gly Gln Gly Trp Gly Leu Asp Ile Ala Thr Val Asn Asn His Tyr Asn
180 185 190

Arg Leu Ile Asn Leu Ile His Arg Tyr Thr Xaa His Cys Leu Asp Thr
195 200 205

Tyr Asn Gln Gly Leu Glu Asn Leu Arg Gly Thr Asn Thr Arg Gln Trp
210 215 220

Xaa Arg Phe Asn Gln Phe Arg Arg Xaa Leu Thr Leu Thr Val Leu Asp
225 230 235 240

Ile Val Ala Leu Phe Pro Asn Tyr Asp Xaa Arg Xaa Tyr Pro Ile Gln
245 250 255

Thr Ser Ser Gln Leu Thr Arg Glu Ile Tyr Thr Ser Ser Val Ile Glu
260 265 270

Asp Ser Pro Val Ser Ala Asn Ile Pro Asn Gly Phe Asn Arg Ala Glu
275 280 285

Phe Gly Val Arg Pro Pro His Leu Met Asp Phe Met Asn Ser Leu Phe
290 295 300

Val Thr Ala Glu Thr Val Arg Ser Gln Thr Val Trp Gly Gly His Leu
305 310 315 320

Val Ser Ser Arg Asn Thr Ala Gly Asn Xaa Ile Asn Phe Pro Xaa Tyr
325 330 335

Gly Val Phe Asn Pro Gly Gly Ala Ile Trp Ile Ala Asp Glu Asp Pro
340 345 350

Arg Pro Phe Tyr Arg Thr Leu Ser Asp Pro Val Phe Val Arg Gly Gly
355 360 365

Phe Gly Xaa Pro His Tyr Val Leu Gly Leu Arg Gly Val Xaa Phe Gln
370 375 380

Gln Thr Gly Thr Asn His Thr Arg Thr Phe Arg Asn Ser Gly Thr Ile
385 390 395 400

Asp Ser Leu Asp Glu Ile Pro Pro Gln Asp Asn Ser Gly Ala Pro Trp
405 410 415

Asn Asp Tyr Ser His Val Leu Asn His Val Thr Phe Val Arg Trp Pro
420 425 430

Gly Glu Ile Xaa Gly Ser Asp Ser Trp Arg Ala Pro Met Phe Ser Trp
435 440 445

Thr His Arg Ser Ala Xaa Xaa Thr Asn Xaa Ile Xaa Pro Xaa Xaa Ile
450 455 460

Thr Gln Ile Pro Xaa Val Xaa Ala His Xaa Leu Xaa Ser Gly Xaa Thr
465 470 475 480

Val Val Arg Gly Pro Gly Phe Thr Gly Gly Asp Xaa Leu Arg Arg Thr
485 490 495

Xaa Xaa Gly Xaa Phe Ala Xaa Xaa Xaa Val Asn Ile Xaa Gly Xaa Leu
500 505 510

Xaa Gln Arg Tyr Arg Xaa Arg Ile Arg Tyr Ala Ser Thr Thr Xaa Leu
515 520 525

Xaa Xaa Xaa Xaa Xaa Xaa Xaa Gly Xaa Xaa Xaa Xaa Xaa Gly Xaa Phe
530 535 540

Xaa Xaa Thr Met Xaa Xaa Gly Asp Xaa Leu Xaa Xaa Xaa Xaa Phe Xaa
545 550 555 560

Xaa Ala Xaa Xaa Xaa Thr Xaa Phe Xaa Phe Xaa Xaa Xaa Gln Ser Xaa
565 570 575

Phe Thr Xaa Gly Xaa Xaa Xaa Phe Xaa Ser Xaa Xaa Glu Val Tyr Ile
580 585 590

Asp Xaa Xaa Glu Xaa Xaa Pro Xaa Xaa Xaa Thr Phe Glu Ala Glu Xaa
595 600 605

Asp Xaa Glu Arg Ala Gln Xaa Ala Val Asn Ala Leu Phe Thr Ser Xaa
610 615 620

Xaa Gln Xaa Gly Xaa Xaa Thr Xaa Val Thr Xaa Tyr His Ile Asp Gln
625 630 635 640

Val Ser Asn Leu Val Xaa Cys Leu Ser Asp Glu Phe Cys Leu Asp Glu
645 650 655

Xaa Arg Glu Leu Ser Glu Xaa Val His Xaa Ala Xaa Arg Leu Ser Asp
660 665 670

Xaa Arg Asn Leu Leu Gln Asp Pro Asn Phe Xaa Gly Ile Asn Arg Gln
675 680 685

Xaa Asp Xaa Gly Trp Arg Gly Ser Thr Asp Ile Thr Ile Gln Xaa Gly
690 695 700

Asp Asp Val Phe Xaa Glu Asn Tyr Val Thr Leu Pro Gly Thr Phe Asp
705 710 715 720

Glu Cys Tyr Pr Thr Tyr Leu Tyr Gln Xaa Ile Asp Glu Ser Xaa Leu
725 730 735

Xaa Xaa Tyr Thr Arg Tyr Gln Leu Arg Gly Tyr Ile Glu Asp Ser Gln
740 745 750

Asp Leu Glu Ile Tyr Leu Ile Arg Tyr Asn Xaa Xaa His Glu Pro Val
755 760 765

Asn Val Xaa Gly Thr Gly Ser Leu Trp Pro Leu Ser Val Xaa Xaa Xaa
770 775 780

Ile Xaa Xaa Cys Gly Glu Pro Asn Arg Cys Ala Pro His Leu Glu Trp
785 790 795 800

Asn Pro Asp Leu Xaa Cys Ser Cys Arg Asp Gly Glu Xaa Cys Xaa His
805 810 815

His Ser His His Phe Ser Leu Asp Ile Asp Val Gly Cys Thr Asp Leu
820 825 830

Asn Glu Asp Leu Xaa Val Trp Xaa Ile Phe Xaa Ile Xaa Thr Gln Asp
835 840 845

Gly His Ala Arg Leu Gly Asn Leu Glu Phe Leu Glu Glu Xaa Pro Leu
850 855 860

Xaa Gly Glu Ala Leu Xaa Arg Val Xaa Arg Ala Glu Xaa Xaa Trp Arg
865 870 875 880

Asp Xaa Arg Glu Xaa Leu Xaa Leu Glu Thr Asn Ile Val Tyr Xaa Glu
885 890 895

Ala Xaa Glu Ser Val Asp Ala Leu Phe Val Asn Ser Gln Tyr Asp Xaa
900 905 910

Leu Gln Ala Asp Thr Asn Ile Ala Met Ile His Ala Ala Asp Xaa Arg
915 920 925

Val His Arg Ile Arg Glu Ala Tyr Leu Pro Glu Leu Ser Val Ile Pro
930 935 940

Gly Val Asn Xaa Xaa Ile Phe Glu Glu Leu Xaa Gly Arg Ile Phe Thr
945 950 955 960

Ala Xaa Xaa Leu Tyr Asp Ala Arg Asn Val Ile Xaa Asn Gly Xaa Phe
965 970 975

Asn Asn Gly Leu Xaa Cys Trp Asn Val Xaa Gly His Val Asp Val Glu
980 985 990

Glu Gln Asn Asn His Arg Ser Val Leu Val Val Pro Glu Trp Glu Ala
995 1000 1005

Glu Val Ser Gln Glu Val Arg Val Cys Pro Gly Arg Gly Tyr Ile Leu
1010 1015 1020

Arg Val Thr Ala Tyr Xaa Glu Gly Tyr Gly Glu Gly Cys Val Thr Ile
1025 1030 1035 1040

His Glu Xaa Xaa Asn Asn Thr Asp Glu Leu Xaa Phe Ser Asn Cys Xaa
1045 1050 1055

Xaa Glu Xaa Val Tyr Pro Xaa Asn Thr Val Xaa Cys Asn Asp Tyr Xaa
1060 1065 1070

Xaa Asn Xaa Xaa Xaa Xaa Xaa Xaa Ala Xaa Xaa Ser Arg Asn Arg Gly
1075 1080 1085

```
Tyr Asp Glu Xaa Tyr Xaa Ser Asn Ser Ser Xaa Pr  Ala Asp Tyr Ala
    1090                1095                1100

Xaa Val Tyr Glu Glu Xaa Xaa Tyr Thr Asp Gly Xaa Arg Xaa Asn Pro
1105                1110                1115                1120

Cys Glu Xaa Asn Arg Gly Xaa Xaa Xaa Xaa Thr Pro Leu Pro Ala Gly
                1125                1130                1135

Tyr Val Thr Xaa Glu Leu Glu Tyr Phe Pro Glu Thr Asp Xaa Val Trp
            1140                1145                1150

Xaa Glu Ile Gly Glu Thr Glu Gly Thr Phe Ile Val Asp Ser Val Glu
        1155                1160                1165

Leu Leu Leu Met Glu Glu
    1170
```

Claims

1. A process for controlling lepidopteran pests which comprises contacting said pests with a lepidopteran-controlling effective amount of *Bacillus thuringiensis* PS91C2, or spores, crystals, or toxins from said isolate, or mutants thereof which retain activity against lepidopteran pests.

2. The process, according to claim 1, wherein a substantially intact *Bacillus thuringiensis* isolate, or mutant thereof which retains activity against lepidopteran pests, is treated to prolong the pesticidal activity when the substantially intact cell is applied to the environment of a target pest.

3. A composition of matter comprising *Bacillus thuringiensis* PS91C2, or a mutant thereof, or spores or crystals of said isolate, in association with an insecticide carrier.

4. A substantially pure toxin protein wherein said toxin has activity against a lepidopteran pest and has at least one characteristic selected from the group consisting of:

(a) the amino acid sequence of said toxin conforms to the Generic Formula shown in SEQ ID NO. 27;

(b) the amino acid sequence of said toxin is at least 75% homologous with toxin 91C2;

(c) the DNA which encodes said toxin hybridizes with DNA which encodes all or part of protein 91C2;

(d) the DNA which encodes said toxin hybridizes with a probe selected from the group consisting of SEQ ID NO. 10, SEQ ID NO. 12, SEQ ID NO. 14, SEQ ID NO. 16, SEQ ID NO. 18, SEQ ID NO. 20, SEQ ID NO. 22, SEQ ID NO. 24, and SEQ ID NO. 26, and DNA encoding SEQ ID NO. 9, SEQ ID NO. 11, SEQ ID NO. 13, SEQ ID NO. 15, SEQ ID NO. 17, SEQ ID NO. 19, SEQ ID NO. 21, SEQ ID NO. 23, and SEQ ID NO. 25;

(e) said toxin is immunoreactive with an antibody which immunoreacts with toxin 91C2; and

(f) the amino acid sequence of said toxin has an alignment value of at least about 450 with toxin 91C2.

5. The toxin, according to claim 4, wherein said toxin conforms to said Generic Formula shown in SEQ ID NO. 27.

6. The toxin, according to claim 4, wherein the amino acid sequence of said toxin has an alignment value of at least about 450 with toxin 91C2.

7. The toxin, according to claim 4, wherein the DNA encoding said toxin hybridizes with DNA which encodes all or part of toxin 91C2.

8. The toxin, according to claim 4, wherein the DNA which encodes said toxin hybridizes with a probe selected from the group consisting of SEQ ID NO. 10, SEQ ID NO. 12, SEQ ID NO. 14, SEQ ID NO. 16, SEQ ID NO. 18, SEQ ID NO. 20, SEQ ID NO. 22, SEQ ID NO. 24, and SEQ ID NO. 26, and DNA coding for SEQ ID NO. 9, SEQ ID NO. 11, SEQ ID NO. 13, SEQ ID NO. 15, SEQ ID NO. 17, SEQ ID NO. 19, SEQ ID NO. 21, SEQ ID NO. 23, and SEQ ID NO. 25.

9. The toxin, according to claim 4, wherein said toxin is immunoreactive with an antibody which immunoreacts with protein 91C2.

10. The toxin, according to claim 4, wherein said toxin has the amino acid sequence consisting essentially of the sequence shown in SEQ ID NO. 4.

11. An isolated polynucleotide encoding a *Bacillus thuringiensis* toxin as defined in claim 4.

12. An isolated polynucleotide, according to claim 11, wherein said polynucleotide comprises DNA which encodes an amino acid sequence shown in SEQ ID NO. 4.

13. The polynucleotide, according to claim 11, wherein said polynucleotide comprises a nucleotide sequence consisting essentially of the sequence shown in SEQ ID NO. 3.

14. A method for controlling lepidopteran pests, wherein said method comprises contacting said pests with a lepidopteran-controlling effective amount of a toxin as defined in claim 1.

15. A host transformed by a nucleotide sequence encoding a toxin as described in claim 4.

16. The transformed host, according to claim 15, wherein said host is transformed by a nucleotide sequence encoding a toxin protein having the amino acid sequence of SEQ ID NO. 4.

17. The transformed host, according to claim 15, wherein said host is transformed to express the nucleotide sequence of SEQ ID NO. 3.

18. A toxin encoded by a nucleotide sequence obtainable from *Bacillus thuringiensis* PS91C2, and variants thereof, wherein said toxin is active against lepidopteran pests.

```
1
MjNNIQNQCV PYNCLxNPEV EILxEERSTG RLPLDISLSL TRFLLSEFVP
GVGVAFGLFD LIWGFITPSx WSLFLLQIEQ LIEQRIETLE RNRAITTLRG
LADSYEbYbE ALREWE-NPN NAQLREDVRI RFANTDDALI TAINNFTLTS
FEIPLLSVYV QAANLHLSLL RDAVSFGQGW GLDIATVNNH YNRLINLIHR

201
YTjHCLDTYN QGLENLRGTN TRQW-RFNQF RRxLTLTVLD IVALFPNYDo
RuYPIQTSSQ LTREIYTSSV IEDSPVSANI PNGFNRAEFG VRPPHLMDFM
NSLFVTAETV RSQTVWGGHL VSSRNTAGN- INFP.YGVFN PGGAIWIADE
DPRPFYRTLS DPVFVRGGFG zPHYVLGLRG V-FQQTGTNH TRTFRNSGTI

401
DSLDEIPPQD NSGAPWNDYS HVLNHVTFVR WPGEI-GSDS WRAPMFSWTH
RSA--TNoIz Px-ITQIPoV KAH-L-SG-T VVRGPGFTGG DbLRRTz-Go
FA-o-VNI-G -L-QRYRoRI RYASTTzLjb -o-b-G-xb- -GxFxkTMx-
GD-L--xzFx -A-bzToF-F ---QS-FTbG uxuF.SzxEV YIDkbEbbPo

601
-oTFEAE-DK ERAQKAVNAL FTSozQbGbK TzVTzYHIDQ VSNLV-CLSD
EFCLDEKREL SEKVHKAKRL SDxRNLLQDP NFkGINRQoD -GWRGSTDIT
IQxGDDVFKE NYVTLPGTFD ECYPTYLYQK IDESKLK-YT RYQLRGYIED
SQDLEIYLIR YN-KHEpVNV oGTGSLWPLS Vjz-Ix-CGE PNRCAPHLEW

801
NPDL-CSCRD GEKCoHHSHH FSLDIDVGCT DLNEDLzVWb IFKIKTQDGH
ARLGNLEFLE EjPLbGEAL- RVKRAEKKWR DKREKLjLET NIVYKEAKES
VDALFVNSQY DjLQADTNIA MIHAADKRVH RIREAYLPEL SVIPGVNo-I
FEELjGRIFT A--LYDARNV IKNGzFNNGL -CWNVKGHVD VEEQNNHRSV

1001
LVVPEWEAEV SQEVRVCPGR GYILRVTAYK EGYGEGCVTI HEbxNNTDEL
KFSNC-xEjV YPzNTVuCND Y--N-X..-Z A--SRNRGYD E-YxSNSSbP
ADYA-VYEEj -YTDGjRzNP CE-NRG...- TPLPAGYVT- ELEYFPETD-
VWbEIGETEG TFIVDSVELL LMEE
```

Figure 1